PTO/SB/21 (01-08)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	10/018,446; Patent No. 6,642,210
Filing Date	21 June 2000; Issued 4 Nov. 2003
First Named Inventor	Jeff Zablocki
Art Unit	1623
Examiner Name	CRANE, LAWRENCE E.
Total Number of Pages in This Submission: 65	Attorney Docket Number: 99-0423-S

ENCLOSURES *(Check all that apply)*

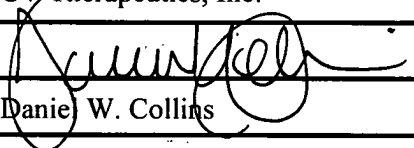
- [X] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [X] Other Enclosure(s) (please Identify below):

Application for Extension of Patent Term Under 35 USC 156 (Original plus 2 copies)

Remarks

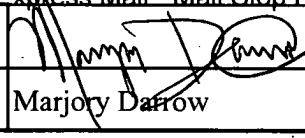
RECEIVED
JUN 06 2008
PATENT EXTENSION
OPLA

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	CV Therapeutics, Inc.		
Signature			
Printed name	Daniel W. Collins		
Date	June 6, 2008	Reg. No.	31,912

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being ~~facsimile transmitted to the USPTO or~~ deposited with the United States Postal Service with sufficient postage as ~~first class mail~~ in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below: Express Mail - Mail Stop Hatch-Waxman PTE

Signature			
Typed or printed name	Marjory Darrow	Date	06/06/2008

PTO/SB/17 (10-07)
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Effective on 12/08/2004.
Fees pursuant to the Consolidated Appropriations Act, 2005 (H.R. 4818).

FEE TRANSMITTAL
For FY 2008

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 1,120.00

Complete if Known	
Application Number	10/018,446; Patent No. 6,642,210
Filing Date	21 June 2000; Issued 4 Nov. 2003
First Named Inventor	Jeff Zablocki
Examiner Name	CRANE, LAWRENCE E.
Art Unit	1623
Attorney Docket No.	99-0423-S

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METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☒ Deposit Account Deposit Account Number: 50-1789 Deposit Account Name: CV Therapeutics, Inc.

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☒ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☒ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☒ Credit any overpayments

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FEE CALCULATION

1. BASIC FILING, SEARCH, AND EXAMINATION FEES

Application Type	FILING FEES Fee ($)	FILING FEES Small Entity Fee ($)	SEARCH FEES Fee ($)	SEARCH FEES Small Entity Fee ($)	EXAMINATION FEES Fee ($)	EXAMINATION FEES Small Entity Fee ($)	Fees Paid ($)
Utility	310	155	510	255	210	105	
Design	210	105	100	50	130	65	
Plant	210	105	310	155	160	80	
Reissue	310	155	510	255	620	310	
Provisional	210	105	0	0	0	0	

2. EXCESS CLAIM FEES

Fee Description	Fee ($)	Small Entity Fee ($)
Each claim over 20 (including Reissues)	50	25
Each independent claim over 3 (including Reissues)	210	105
Multiple dependent claims	370	185

Total Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - 20 or HP =	_____	x _____	= _____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - 3 or HP =	_____	x _____	= _____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims Fee ($)	Multiple Dependent Claims Fee Paid ($)

3. APPLICATION SIZE FEE

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $260 ($130 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
_____ - 100 =	_____ / 50 =	_____ (round up to a whole number)	x 260.00	= 0.00

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): Hatch-Waxman - Patent Term Extension	1,120.00

SUBMITTED BY

Signature	[signature]	Registration No. (Attorney/Agent) 31,912	Telephone 650-384-8047
Name (Print/Type)	Daniel W. Collins		Date June 6, 2008

PTO/SB/17 (10-07)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Art Unit	1623
Attorney Docket No.	99-0423-S

RECEIVED
JUN 06 2008
PATENT EXTENSION
OPLA

METHOD OF PAYMENT (check all that apply)

☐ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☒ Deposit Account Deposit Account Number: 50-1789 Deposit Account Name: CV Therapeutics, Inc.

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

☒ Charge fee(s) indicated below ☐ Charge fee(s) indicated below, **except for the filing fee**

☒ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☒ Credit any overpayments

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1. BASIC FILING, SEARCH, AND EXAMINATION FEES

	FILING FEES		SEARCH FEES		EXAMINATION FEES		
		Small Entity		Small Entity		Small Entity	
Application Type	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fee ($)	Fees Paid ($)
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_____ - 20 or HP =	_____ x	_____ =	_____

HP = highest number of total claims paid for, if greater than 20.

Indep. Claims	Extra Claims	Fee ($)	Fee Paid ($)
_____ - 3 or HP =	_____ x	_____ =	_____

HP = highest number of independent claims paid for, if greater than 3.

Multiple Dependent Claims	
Fee ($)	Fee Paid ($)
_____	_____

3. APPLICATION SIZE FEE

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Total Sheets	Extra Sheets	Number of each additional 50 or fraction thereof	Fee ($)	Fee Paid ($)
_____ - 100 =	_____ / 50 =	_____ (round up to a whole number) x	260.00 =	0.00

4. OTHER FEE(S)

	Fees Paid ($)
Non-English Specification, $130 fee (no small entity discount)	
Other (e.g., late filing surcharge): Hatch-Waxman - Patent Term Extension	1,120.00

SUBMITTED BY

Signature		Registration No. (Attorney/Agent) 31,912	Telephone 650-384-8047
Name (Print/Type)	Daniel W. Collins		Date June 6, 2008

This collection of information is required by 37 CFR 1.136. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

In re:	US Patent No. 6,642,210
Issued:	November 4, 2003
Application No:	10/018,446
Filed:	June 21, 2000
Inventors:	Zablocki et al.
Assignee:	CV Therapeutics, Inc.
For:	2-(N-pyrazolo)adenosines with application as adenosine A_{2A} receptor agonists

Mail Stop Hatch-Waxman PTE
Director of the United States Patent and Trademark Office
PO Box 1450
Alexandria, Virginia 22331-1450

Application for an extension of patent term under 35 USC 156

CV Therapeutics, Inc., a Delaware corporation, is the assignee of the entire interest in US Patent No. 6,642,210, issued on November 4, 2003, for 2-(N-pyrazolo)adenosines with application as adenosine A_{2A} receptor agonists, by an assignment from the inventors, Zablocki et al., to CV Therapeutics, Inc. recorded on September 1, 2004, at Reel 015056, Frame 0942.

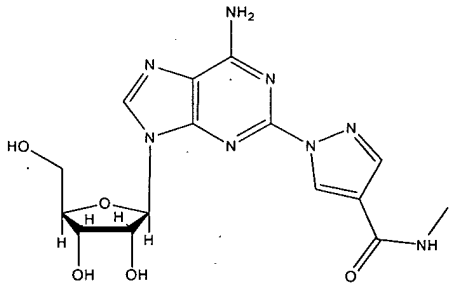
CV Therapeutics, Inc. hereby requests an extension of the patent term of US Patent No. 6,642,210 by stating that the regulatory review period has been completed, and by providing the following information, as required by 35 USC 156 and 37 CFR 1.710.

1. Complete identification of product

The product recently approved by the US Food and Drug Administration is Lexiscan™ regadenoson 0.4 mg/5 mL injectable solution.

It comprises a compound having:

(a) the structural formula:



(b) the molecular formula: $C_{15}H_{18}N_8O_5$;

(c) the molecular weight: 390.35;

(d) the chemical names:

(1) adenosine, 2-[4-[(methylamino)carbonyl]-1*H*-pyrazol-1-yl]-; and

(2) 1-[6-amino-9-[(2R,3R,4S,5R)-3,4-dihydroxy-5- (hydroxymethyl)oxolan-2-yl]purin-2-yl]- N-methylpyrazole-4-carboxamide; and

(3) (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide [from US Patent No. 6,642,210 e.g. at claim 8, column 17, lines 54-59]; and

(4) 2-(4-methylaminocarbonylpyrazol-1-yl)adenosine [from US Patent No. 6,403,567 e.g. at claim 7].

(e) the generic names:

(1) regadenoson (monohydrate) (USAN), and

(2) regadenoson (monohydrate) (INN); and

(f) the CAS registry numbers:

(1) 313348-27-5 (free base); and

(2) 875148-45-1 (monohydrate).

2. Identification of Federal statute/provision of law

Lexiscan ™ (regadenoson 0.4 mg/5 mL injectable solution), was subject to regulatory review under Section 505(b) of the Federal Food, Drug and Cosmetic Act.

3. Date on which product received permission for commercial marketing or use

Lexiscan ™ (regadenoson 0.4 mg/5 mL injectable solution), received permission for commercial marketing under Section 505 (b) of the Federal Food, Drug and Cosmetic Act on April 10, 2008.

4. Identification of active ingredient

Lexiscan ™ (regadenoson 0.4 mg/5 mL injectable solution), contains as its sole active ingredient regadenoson monohydrate, which in solution is converted to free base, described above in item 1. This product has not previously been approved for commercial marketing under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act or the Virus-Serum-Toxin Act.

5. Time period for submitting application

This application for extension of patent term is being submitted within the sixty day period permitted for submission pursuant to Section 37 CFR 1.720 (f). The date of FDA approval was April 10, 2008. The submission period begins on April 10, 2008, and ends on June 8, 2008.

6. Identification of patent

The patent for which patent term extension is being sought is US Patent No. 6,642,210 inventors Zablocki et al., which issued on November 4, 2003, for N 2-(N-PYRAZOLO)ADENOSINES WITH APPLICATION AS ADENOSINE A_{2A} RECEPTOR AGONISTS. The term of US Patent No. 6,642,210 will expire, unless extended again, on June 22, 2019.

7. Copy of patent

A copy of US Patent No. 6,642,210 is attached as Attachment A.

8. Other patent documents

A copy of the Certificate of Correction which issued with respect to US Patent No. 6,642,210 is attached as Attachment B.

Notice of Recordation and assignment from the inventors to CV Therapeutics, Inc. is attached as Attachment C.

A copy of the Terminal Disclaimer filed with respect to related US Patent No. 6,403,567 is attached as Attachment D.

No Reexamination Certificate has issued with respect to US Patent No. 6,642,210.

9. Claims covering the product

US Patent No. 6,642,210 claims Lexiscan™ (regadenoson 0.4 mg/5 mL injectable solution), in the following applicable claim:

Claims 1, 3, 6 cover regadenoson free base when

R^3 is $CONR^7R^8$, where R^7 is methyl and R^8 is hydrogen.

Claims 8 and 13 cover regadenoson free base **(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide**.

Claims 9 and 10 cover the use of regadenoson free base for use in stimulating coronary vasodilation in a mammal for purposes of imaging the heart when

R^3 is $CONR^7R^8$, where R^7 is methyl and R^8 is hydrogen.

Claim 11 covers the pharmaceutical formulation Lexiscan™ when

R^3 is $CONR^7R^8$, where R^7 is methyl and R^8 is hydrogen,

and when the formulation contains a pharmaceutically acceptable excipient such as propylene glycol.

Claim 12 covers the pharmaceutical formulation Lexiscan™ when

R^3 is $CONR^7R^8$, where R^7 is methyl and R^8 is hydrogen,

and when the formulation is in the form of a solution and contains a pharmaceutically acceptable excipient such as propylene glycol.

10. Relevant dates and information pursuant to 35 USC 156(g)

The relevant dates and information under 35 USC 156(g) and 37 CFR 1.740(10)(i) are as follows:

August 2, 2001:	Effective date of IND 62,862;
May 14, 2007:	Submission date of NDA 22-161.
April 10, 2008:	Approval date of NDA 22-161.

11. Brief description of significant activities

A brief description of the activities undertaken during the regulatory review period follows.

Submission No.	Date	Content
IND 62,862	6/29/01	Submission of IND application
IND 62,862	7/10/01	Acknowledgement of receipt of IND application
IND 62,862	7/13/01	Fax submission of additional CMC information
IND 62,862	8/24/01	Transmittal of Advertisements and Promotional Labeling for Drugs and Biologics for Human Use: (Print Press Release)
IND 62,862	8/28/01	Submission of investigator information in CVT 5121
IND 62,862	10/24/01	Submission of amended protocol CVT 5121
IND 62,862	11/20/01	Fax from FDA containing nonclinical comments
IND 62,862	12/13/01	Submission of investigator information in CVT 5121
IND 62,862	1/10/02	Submission of investigator information in CVT 5121
IND 62,862	1/18/02	Voicemail regarding change in CVT contacts for projects
IND 62,862	1/31/02	Submission of change in primary contact for IND 62,862
IND 62,862	2/6/02	Submission of investigator information in CVT 5121
IND 62,862	4/5/02	Submission of investigator information in CVT 5121
IND 62,862	5/3/02	Fax submission of meeting request
IND 62,862	5/10/02	Fax meeting request and list of questions
IND 62,862	5/21/02	Fax from FDA confirming meeting
IND 62,862	5/29/02	Submission of faxed meeting request and list of questions
IND 62,862	7/3/02	Submission of clinical development plan package
IND 62,862	7/23/02	Fax list of CVT meeting attendees
IND 62,862	7/26/02	Minutes from 26 Jul 02 meeting
IND 62,862	7/29/02	Submission of change in authorized representative for IND 62,862
IND 62,862	7/30/02	Submission of new protocol CVT 5122; submission of investigator information in CVT 5121; submission of copy of fax dated 7/23/02
IND 62,862	8/7/02	Submission of CMC information
IND 62,862	8/21/02	Submission of investigator information in CVT 5122
IND 62,862	8/22/02	Fax regarding overheads used by CVT for the presentation at the 26 Jul 02 meeting
IND 62,862	8/28/02	Fax regarding overheads used by CVT for the presentation at the 26 Jul 02 meeting. Resent, as one overhead was not sent in the facsimile dated 22 Aug 02.
IND 62,862	9/4/02	Submission of CMC information
IND 62,862	9/11/02	Submission of Initial Written Report for study CVT 5122
IND 62,862	9/19/02	Submission of investigator information in CVT 5121, and CVT 5122; submission of copy of CVT facsimile dated 28 Aug 02: Presentation overheads at 26 Jul 02 meeting.
IND 62,862	9/23/02	Minutes of a voicemail regarding new CSO, Russell Fortney
IND 62,862	9/24/02	Submission of 1st Annual Progress Report
IND 62,862	10/4 & 9/02	Letter from FDA re 30 Jul 02 protocol
IND 62,862	10/9/02	Fax of FDA meeting minutes of 26 Jul 02
IND 62,862	10/16/02	Submission of investigator information in CVT 5121
IND 62,862	11/13/02	Submission of investigator information in CVT 5121 and CVT 5122
IND 62,862	12/16/02	Submission of protocol amendment to CVT 5121; submission of protocol amendment to CVT 5122; submission of investigator information in CVT 5122
IND 62,862	2/5/03	Submission of investigator information in CVT 5121

Submission No.	Date	Content
IND 62,862	4/2/03	Submission of protocol amendment to CVT 5122
IND 62,862	4/14/03	Submission of Follow-Up Written Report for study CVT 5122
IND 62,862	4/25/03	Submission response regarding mouse bone marrow micronucleus assay
IND 62,862	5/2/03	Submission request for FDA End-of-phase II meeting
IND 62,862	5/2/03	Fax requesting a meeting
IND 62,862	5/8 & 9/03	Voicemail regarding tentative date selected for End of Phase 2 meeting
IND 62,862	5/12/03	Fax confirming End of Phase 2 meeting
IND 62,862	5/13/03	Phone inquiry as to if Imaging Division representatives should be included in the EOP2 meeting
IND 62,862	6/16-17/03	Voicemail requesting twenty copies of the End of phase 2 briefing package
IND 62,862	6/17/03	Submission of information amendment; submission of reports 3003-004P, 3003-004, 3003-005P, and 3003-005
IND 62,862	6/20/03	Submission regarding End of Phase 2 meeting information package
IND 62,862	7/8/03	Voicemail regarding questions for the 11 Jul 03 meeting
IND 62,862	7/9/03	Email regarding list of FDA meeting attendees
IND 62,862	7/11/03	Minutes-CVT: CVT 3146 End of Phase 2 Meeting
IND 62,862	7/18/03	Phone, feedback on the use of radionuclide in the Phase 3 study
IND 62,862	7/23-24/03	Phone regarding dual isotope protocol acceptable; teleconference date to be determined.
IND 62,862	7/25/03	Fax of FDA End of Phase 2 meeting minutes of 11 Jul 03
IND 62,862	7/30/03	Fax regarding teleconference information for 31 Jul 03
IND 62,862	7/31/03	Teleconference follow-up to the 11 Jul 03 EOP2 meeting
IND 62,862	8/5-6/03	Email regarding teleconference arrangements, list of teleconference participants, and request for electronic copies of references
IND 62,862	8/6/03	Teleconference minutes between CVT, FDA, and University of Alabama
IND 62,862	8/7/03	Fax of FDA minutes of 31 Jul 03, and 06 Aug 03 teleconferences
IND 62,862	8/12/03	Fax of corrected FDA minutes of 06 Aug 03 teleconference
IND 62,862	9/4/03	Fax of proposed analysis plan for secondary endpoints in CVT 5131, and complete analysis plan from CVT 5131
IND 62,862	9/9/03	Fax regarding AT & T teleconference information for 12 Sep 03
IND 62,862	9/12/03	Teleconference discussion of analysis of secondary endpoints
IND 62,862	9/16/03	Submission of information amendment to CVT 3146; submission of reports 0793-7771, 0794-7771, 124-021, 124-022, 124-023, and 124-020
IND 62,862	9/26/03	Fax of FDA teleconference minutes of 12 Sep 03, discussion of analysis of secondary endpoints
IND 62,862	10/1/03	Submission of protocol amendment to CVT 5131, and transfer of obligations to CROs
IND 62,862	10/24/03	Submission of investigator information in CVT 5131
IND 62,862	10/30/03	Submission of 2nd Annual Progress Report
IND 62,862	11/26/03	Submission protocol amendment to CVT 5131; submission of investigator information in CVT 5131, and CVT 5121; submission of CVT faxes
IND 62,862	1/7/04	Submission of investigator information to CVT 5131
IND 62,862	1/21/04	Submission of statistical analysis plan for CVT 5131; submission of CVT 5131 Mockup Tables, Figures, and Listings
IND 62,862	2/3/04	Submission of protocol amendment to CVT 5131; submission of investigator information in CVT 5131
IND 62,862	2/6/04	Submission of new protocol CVT 5132; submission of transfer of obligations to CROs for CVT 5132; submission of CMC information
IND 62,862	2/12/04	Phone follow-up
IND 62,862	2/27 & 3/2/04	Email regarding review of the Statistical Analysis Plan, the Pulmonary Division still has not finished with the questions regarding the COPD/asthma study

Submission No.	Date	Content
IND 62,862	3/3/04	Submission of investigator information to CVT 5131
IND 62,862	3/3 & 9/04	Email regarding reference nos. 43, 44, 45, and 47 from the briefing package (11 Jul 03 meeting) sent to FDA as requested
IND 62,862	3/11/04	Email regarding no comments for submissions 034 and 036 from Dr. Hung, and still awaiting on comments from the Pulmonary Division
IND 62,862	3/31/04	Submission protocol amendment to CVT 5121; submission of investigator information to CVT 5131
IND 62,862	3/31/04	Email response from the Pulmonary Division on COPD/asthma study design from the 11 Jul 03 meeting
IND 62,862	4/14/04	Submission protocol amendments to CVT 5131 and CVT 5132
IND 62,862	4/19/04	Submission request for End-of-Phase 2 Meeting to discuss CMC aspects of regadenoson development program, and to review the toxicology studies conducted for the program
IND 62,862	4/19/04	Fax copy of cover letter (#0040): Request for End-of-Phase 2 Meeting
IND 62,862	4/26/04	Email confirmation of Type B Meeting (End of Phase 2) for 18 Jul 04
IND 62,862	4/28/04	Submission of investigator information to CVT 5131; submission of investigator information to CVT 5132
IND 62,862	5/4-6/04	Voicemail and phone conversation regarding Dr. Stockbridge not attending the EOP2 meeting, request of a copy of the briefing materials for the two pharmacology reviewers, and draft questions and toxicology section for pharmacology reviewers sent via fax
IND 62,862	5/5/04	Fax of draft Information Package of 18 May 04 for Pharmacology/Toxicology reviewers
IND 62,862	5/10/04	Submission of Information Package for the 18 May 04 End of Phase 2 Meeting
IND 62,862	5/13/04	Phone call notifying CVT that one FDA chemist will not be able to attend the 18 May 04 meeting, rescheduling of EOP2 meeting will not be necessary
IND 62,862	5/14/04	Fax requesting for FDA feedback on the proposal to modify ECG measurements for protocols CVT 5131 and CVT 5132
IND 62,862	5/18/04	Minutes-CVT: CVT-3146 End of Phase 2 Meeting
IND 62,862	5/24, 6/24, 6/14-17/04	Phone and voicemail regarding storage configurations to be used in CVT's stability protocols for sterility and endotoxin test samples
IND 62,862	5/26/04	Submission of investigator information to CVT 5131 and CVT 5132; submission of technical report 1491/CVT/01-B
IND 62,862	6/1/04	Voicemail regarding status of FDA review/response on CVT's ECG proposal sent by fax, feedback from the FDA microbiologist on the adequacy of the 15-minute sterilization cycle, outcome of discussion on the adequacy of the toxicology qualification studies conducted
IND 62,862	6/2/04	Email agreement on proposal to modify the ECG measurements in protocols CVT 5131 and CVT 5132
IND 62,862	6/3-4/04	Email request for information regarding the toxicology qualification studies conducted
IND 62,862	6/4/04	Voicemail regarding information in the EOP2 briefing package
IND 62,862	6/4/04	Fax requesting feedback on revised clinical study design to evaluate the effect of caffeine on CVT-3146-induced increase in coronary blood flow
IND 62,862	6/15/04	Phone FDA requesting for information regarding the drug substance lots used in the 28-day toxicology study
IND 62,862	6/17/04	Minutes-FDA (Email): FDA End of Phase 2 Meeting Minutes of 18 May 04
IND 62,862	6/18, 6/21-22/04	Email request and confirmation of teleconference for 04 Aug 04

Submission No.	Date	Content
IND 62,862	6/21/04	Submission of investigator information in CVT 5131 and CVT 5132; submission of technical reports 6892-108, AA89JT.503.BTL, CVT3146.053-P: submission of faxes requesting feedback
IND 62,862	6/21/04	Submission request for teleconference to discuss CMC information
IND 62,862	6/21/04	Fax copy of cover letter (#0045): Request for teleconference
IND 62,862	6/23/04	Email confirmation of teleconference for 04 Aug 04
IND 62,862	6/24/04	Email timing for teleconference briefing package and clinical reviewer for ECG question and caffeine study
IND 62,862	6/30/04	Phone comments on Caffeine Study (CVT 5123) Synopsis
IND 62,862	7/1/04	Minutes-CVT (Teleconference): Discussion of Caffeine Study (CVT 5123) Synopsis
IND 62,862	7/2/04	Submission of protocol amendments in CVT 5131 and CVT 5132
IND 62,862	7/14/04	Email regarding Microbiologist comment on sterilization cycle
IND 62,862	7/23/04	Submission of Information Package for 04 Aug 04 Teleconference
IND 62,862	7/30/04	Submission of new protocol CVT 5112; submission of investigator information in CVT 5131 and CVT 5132
IND 62,862	8/5/04	Minutes-CVT: Discussion of impurity
IND 62,862	8/13/04	Phone regarding comments on Protocol CVT 5112 – Renal Study
IND 62,862	8/16/04	Minutes-FDA (Email): Meeting minutes of 05 Aug 04 teleconference
IND 62,862	8/18/04	Submission of investigator information in CVT 5131 and CVT 5132
IND 62,862	8/24/04	Submission of new protocol CVT 5123
IND 62,862	9/2/04	Letter regarding Protocol CVT 5123
IND 62,862	9/7/04	Email of letter regarding Protocol CVT 5123
IND 62,862	9/13, 9/17, 9/21/04	Phone discussion regarding FDA recommendation in the 02 Sep 04 letter that caffeine use not be restricted in the efficacy trails, and clarification regarding Dr. Peter Hinderling's review of Protocol CVT 5123
IND 62,862	9/15/04	Phone request for references for Protocol CVT 5123
IND 62,862	9/16/04	Submission of investigator information in CVT 5131 and CVT 5132
IND 62,862	9/17/04	Submission response to FDA request for copies of references cited in Protocol CVT 5123
IND 62,862	9/27/04	Submission of protocol amendment to CVT 5112
IND 62,862	9/29/04	Email regarding Caffeine Interaction Study response status
IND 62,862	10/5/04	Submission of Request for Waiver of Pediatric Studies
IND 62,862	10/11/04	Submission of 3rd Annual Progress Report: 01 Jun 03 – 31 May 04
IND 62,862	10/12/04	Letter regarding review of Protocol CVT 5123 (#0050) completed, with comments and recommendations
IND 62,862	10/13/04	Email new letter (12 Oct 04) regarding the caffeine study (CVT 5123/#0050): Comments and recommendations
IND 62,862	10/22/04	Letter granting the pediatric waiver
IND 62,862	10/25/04	Email of letter (22 Oct 04) granting the pediatric waiver
IND 62,862	10/28/04	Submission of investigator information in CVT 5131 and CVT 5132
IND 62,862	11/12/04	Submission response to the 12 Oct 04 letter from the Division containing comments and recommendations concerning proposed Study CVT 5123
IND 62,862	11/17-18/04	Email follow-up to review of response on Protocol CVT 5123
IND 62,862	11/19/04	Email of teleconference confirmation (10 Dec 04) to discuss caffeine interaction protocol (CVT 5123)
IND 62,862	11/24, 11/26/04	Email regarding teleconference re-scheduled for 14 Dec 04
IND 62,862	12/8-9/04	Email follow-up on teleconference to discuss the caffeine study, and comment from FDA internal meeting
IND 62,862	12/13/04	Submission of new protocol CVT 5124
IND 62,862	12/14/04	Minutes-CVT: Discussion of any additional comments from the Agency on the protocol (CVT 5123) after their review of CVT's responses

Submission No.	Date	Content
IND 62,862	12/16/04	Submission of investigator information in CVT 5112, CVT 5131, CVT 5132, and transfer of obligation to a CRO for Study CVT 5112
IND 62,862	12/17/04	Phone regarding Protocol CVT 5124 acceptable to proceed with the study
IND 62,862	1/11/05	Minutes-FDA (Email): Meeting minutes of teleconference on 14 Dec 04
IND 62,862	1/21/05	Submission of protocol amendment to CVT 5123; submission of investigator information in CVT 5131, CVT 5132, and transfer of obligation to CRO for Study CVT 5132
IND 62,862	2/17/05	Submission of investigator information in CVT 5131, CVT 5132, and transfer of obligation to CRO for Study 5131
IND 62,862	3/16/05	Submission of investigator information in CVT 5123, CVT 5124, CVT 5131, CVT 5132, and transfer of obligation to CRO's for CVT 5123 and 5124
IND 62,862	4/15/05	Submission of investigator information in CVT 5131, CVT 5132, and transfer of obligation to CRO for CVT 5131
IND 62,862	5/11/05	Submission of investigator information in CVT 5131 and CVT 5132
IND 62,862	6/8/05	Submission of investigator information in CVT 5131, CVT 5132, and transfer of obligation to CRO for Study CVT 5131
IND 62,862	7/13/05	Submission of investigator information in CVT 5124, CVT 5131 and CVT 5132
IND 62,862	7/24/05	Fax of proposed change to Protocol CVT 5131
IND 62,862	7/24/05	Email follow-up to fax sent 24 Jul 05, copy of fax attached, and Study CVT 5131 protocol Amendment IV attached
IND 62,862	8/4/05	Email response to fax on 24 Jul 05
IND 62,862	8/11/05	Submission of protocol amendment to CVT 5131; submission of investigator information in CVT 5131 and CVT 5132
IND 62,862	8/22/05	Submission of protocol amendment to CVT 5131
IND 62,862	8/22-23/05	Email regarding copy of request for feedback, and status of request for feedback
IND 62,862	8/23/05	Phone regarding clarification on Protocol Amendment for CVT 5131
IND 62,862	8/24/05	Email of Dr. Hung's comments regarding the proposed protocol amendment for CVT 5131
IND 62,862	8/25/05	Fax response to request for information regarding the proposed protocol amendment for CVT 5131
IND 62,862	8/25/05	Email copy of the faxed response to request for information regarding the proposed protocol amendment for CVT 5131
IND 62,862	8/29, 9/1/05	Email status of review of proposed protocol CVT 5131 change
IND 62,862	9/7/05	Email response to fax on 25 Aug 05 regarding the protocol amendment changes for CVT 5131
IND 62,862	10/12/05	Submission of 4th Annual Progress Report
IND 62,862	11/10/05	Submission of investigator information in CVT 5131; submission of technical reports CVT3146.017-R, CVT3146.024-R,a and CVT3146.025-R
IND 62,862	12/14/05	Submission of investigator information in CVT 5131
IND 62,862	12/21/05	Submission of protocol amendment to CVT 5131
IND 62,862	1/23/06	Submission of protocol amendment to CVT 5125
IND 62,862	2/23/06	Submission of investigator information in CVT 5125 and CVT 5131; submission of technical reports CVT3146.117-P, CVT3146.118-P, CVT3146.122-P, CVT3146.125-P, CVT3146.001-N, CVT3146.008-MET, CVT3146.009-MET, CVT3146.010-MET, CVT3146.016-R, CVT3146.026-R, CVT3146.046-N, CVTTOX#04-005, CVT3146.028-T, and transfer of obligation to CRO for Study CVT 5125
IND 62,862	4/4/06	Submission requesting feedback regarding commercial acceptance criteria for drug substance impurities CVT-3145 and N6-methyl CVT-3146

Submission No.	Date	Content
IND 62,862	4/6/06	Email notification of submission 0075 sent 04 Apr 06 regarding a request for feedback
IND 62,862	4/14/06	Submission of protocol amendment for CVT 5126; submission of investigator information in CVT 5126 and CVT 5131
IND 62,862	4/25/06	Phone requesting status of feedback on Pharm/Tox question and identity of new Pharm/Tox reviewer
IND 62,862	5/1/06	Phone explanation of CVT 5132 Data Correction Plan
IND 62,862	5/5, 5/8/06	Email requesting status on the request for feedback (04 Apr 06) from the Pharm/Tox reviewer, and response to request for status on request for feedback (04 Apr 06) from the Pharm/Tox reviewer
IND 62,862	5/9/06	Email request for definition of area % in tables submitted in the request for feedback (04 Apr 06), definition of area % provided in response to the request
IND 62,862	5/19/06	Email response to request for feedback from Pharm/Tox reviewer (04 Apr 06)
IND 62,862	6/9/06	Submission of protocol amendment for CVT 5125; submission of investigator information for CVT 5125. CVT 5126, and CVT 5131
IND 62,862	6/14/06	Submission of Pre-NDA teleconference request – Chemistry and Pharmacology
IND 62,862	6/14/06	Email cover letter and Form FDA 1571 for Serial No. 078
IND 62,862	6/16/06	Submission response to FDA comments in 18 May 06 email, and request for teleconference
IND 62,862	6/18-20/06	Email of cover letter and Form FDA 1571 for Serial No. 0079, scheduling and confirmation of Pre-NDA teleconference
IND 62,862	6/23/06	Submission outline of errors in Study CVT 5132 and plan for correcting the data, and request for feedback
IND 62,862	6/25/06	Email of cover letter and Form FDA 1571 for Serial No. 0080
IND 62,862	6/28/06	Phone regarding question on impurity feedback, confirmation of receipt of email, and timing estimate for NDA and other meetings
IND 62,862	6/29/06	Submission of Pre-NDA CMC-Pharm/Tox Information Package
IND 62,862	6/30/06	Email of E-copy of Serial No. 0081 (Information Package), and confirmation of receipt
IND 62,862	7/3/06	Email question regarding impurities
IND 62,862	7/6/06	Phone regarding transfer of regadenoson project within CDER from DCRP to Division of Medical Imaging and Hematology Products
IND 62,862	7/11/06	Phone follow-up on change in Division responsibility for regadenoson
IND 62,862	7/11-12/06	Phone regarding transfer of IND to Division of Medical Imaging and Hematology Products
IND 62,862	7/12/06	Phone regarding transfer of IND to Division of Medical Imaging and Hematology Products
IND 62,862	7/13/06	Voicemail regarding transfer of IND to Division of Medical Imaging and Hematology Products
IND 62,862	7/14/08	Letter regarding pending transfer of file from DCRP to DMIHP
IND 62,862	7/14/06	Phone follow-up on CVT contacts with the Agency regarding transfer of the IND
IND 62,862	7/14, 7/21/06	Email E-copy of letter regarding pending transfer of file from DCRP to DMIHP, and acknowledgement by Jenkins of CVT discussions with OND staff regarding pending transfer
IND 62,862	7/17/06	Phone follow-up on change in Division responsibility for regadenoson
IND 62,862	7/19-20/06	Phone regarding transfer of IND to DMIHP
IND 62,862	7/20/06	Phone regarding transfer of IND to DMIHP
IND 62,862	7/20/06	Phone regarding pending transfer of file from DCRP to DMIHP

Submission No.	Date	Content
IND 62,862	7/21/06	Email contact information for the week
IND 62,862	7/26-27/06	Email of DCRP meeting minutes from 25 Jul 06 preliminary pre-NDA meeting
IND 62,862	7/27/06	Letter pending transfer of file from DCRP to DMIHP
IND 62,862	7/27-28/06	Email E-copy of the letter responding to communications regarding the pending transfer of file from DCRP to DMIHP, acknowledgement of response from DMIHP
IND 62,862	7/27/06	Phone follow-up on pre-NDA CMC meeting regarding the question on the Division response to question 6 in the Information Package
IND 62,862	8/3/06	Submission of protocol amendment to CVT 5125
IND 62,862	8/9/06	Email feedback on impurity question
IND 62,862	8/29/06	Email of final response to the 0079 submission re: impurities N6-Methyl CVT-3146 and CVT-3145
IND 62,862	9/25-26/06	Email of confirmation of Division transfer, contact in DMIHP
IND 62,862	9/28/06	Email of confirmation of address for IND submission
IND 62,862	9/29/06	Submission of 5th Annual Progress Report: 01 Jun 05 – 31 May 06
IND 62,862	10/27/06	Submission of protocol amendment to CVT 5126; submission of investigator information to CVT 5126; submission of technical report CVT3146.050-N, and technical summaries for CVT3146.050-N, CVT3146.112-P, CVT3146.124-P, CVT3146.128-P, CVT3146.129-P, CVT3146.130-P, and CVT3146.132-P
IND 62,862	11/2/06	Phone regarding Regadenoson regulatory project manager
IND 62,862	11/10/06	Email E-copy of submission 0085: Request for Pre-NDA Meeting (Type B): Clinical
IND 62,862	11/10/06	Submission of request for Pre-NDA Meeting (Type B): Clinical
IND 62,862	11/14, 11/20/06	Phone of Pre-NDA meeting request - Clinical
IND 62,862	11/20/06	Email of scheduling of the Pre-NDA meeting – Clinical
IND 62,862	11/27/06	Letter of request for resubmission of Pre-NDA meeting request and a brief description of Phase 3 primary endpoints
IND 62,862	11/29, 12/4-5/06	Phone regarding scheduling Clinical pre-NDA meeting
IND 62,862	12/4/06	Submission of Pre-NDA meeting request (Type B): Clinical
IND 62,862	12/4/06	Fax of copy of Pre-NDA meeting request (Type B): Clinical
IND 62,862	12/11/06	Phone regarding scheduling of the Pre-NDA meeting
IND 62,862	12/12/06	Phone regarding Regadenoson pre-NDA meeting
IND 62,862	12/15/06	Phone regarding dates for End of Phase 3 and Pre-NDA meetings
IND 62,862	12/18/06	Letter and fax regarding IND 62,862 (Regadenoson)/EOP3 and Pre-NDA meeting granted letter
IND 62,862	12/18-20/06	Phone regarding details on End of Phase 3 and Pre-NDA meetings
IND 62,862	1/3/07	Submission of Information Package: End of Phase 3 and Pre-NDA teleconference
IND 62,862	1/23/07	Fax of Pharmacology/Toxicology information request for End of Phase 3 meeting
IND 62,862	1/22-24/06	Phone regarding End of Phase 3, Pre-NDA and Post-Submission meetings and questions on trade name clearance
IND 62,862	1/25/07	Email regarding draft slides for End of Phase 3 meeting and name of additional meeting participant
IND 62,862	1/26/07	Email of request for Pharmacology/Toxicology information
IND 62,862	1/29/07	Email of FDA participants for End of Phase 3 meeting
IND 62,862	1/29/07	Phone regarding FDA participants for End of Phase 3 meeting, comments from the FDA pre-NDA Meeting and questions on trade name clearance
IND 62,862	1/30/07	Email regarding End of Phase 3 meeting Division comments and information requests

Submission No.	Date	Content
IND 62,862	2/3/07	Email regarding copy of slides from End of Phase 3 meeting
IND 62,862	2/5/07	Email regarding FDA/DMIHP comments for Pre-NDA meeting 06 Feb 07 and CVT teleconference participants
IND 62,862	2/9/07	Email regarding Pre-NDA meeting
IND 62,862	2/15/07	Phone requesting NDA number
IND 62,862	2/15/07	Phone regarding General User Fee information
IND 62,862	2/20/07	Phone regarding minutes for EOP3 and Pre-NDA meetings
IND 62,862	2/20-21/07	Email of EOP3 meeting questions
IND 62,862	2/28/07	Phone follow-up on status of EOP3 and Pre-NDA meeting minutes
IND 62,862	2/28/07	Letter Pre-NDA meeting minutes
IND 62,862	3/2/07	Email regarding Efficacy Dataset Specification for Regadenoson NDA
IND 62,862	3/8/07	Letter End of Phase 3 meeting minutes to sponsor
IND 62,862	3/29-30/07	Phone follow-up on test submission and feedback from Dr. Mucci on dataset specifications
IND 62,862	4/3/07	Email regarding Efficacy dataset for NDA to be sent in EXCEL (or equivalent MINITAB) formatted sets in addition to the SAS formatted sets
IND 62,862	4/4-5/07	Email sample eCTD submission (900162) processed without any issues
IND 62,862	4/24/07	Submission request for feedback on Proposed Trade Name LEXISCAN™
IND 62,862	4/24/07	Letter of three desk copies of submission #0088
IND 62,862	9/28/07	Submission of 6th Annual Progress Report: 01 Jun 06 – 31 May 07
IND 62,862	11/8/07	Email regarding Trade name Pre-Clearance
IND 62,862	1/22/08	Submission of technical report CVT3146.149-P, CVT3146.057-T, and CVT3146.056-T
IND 62,862	1/24/08	Letter of Authorization
NDA 22-161	5/4/06	Phone regarding submission of CDISC Study Data Tabulation Model in eCTD format
NDA 22-161	5/4/06	Email STDM (CDISC) Sample Submission
NDA 22-161	7/26/06	Submission of CTD 900130: Sample Study Data Tabulation Model (SDTM)
NDA 22-161	8/23, 8/25, 8/28/06	Email regarding status of sample submission (CTD 900130) review and request for MedDRA dictionary version
NDA 22-161	8/28-29/06	Email regarding status of sample submission (CTD 900130) review
NDA 22-161	9/8, 9/11/06	Email regarding status of sample submission (CTD 900130) review
NDA 22-161	9/12/06	Email regarding feedback regarding sample submission (CTD 900130)
NDA 22-161	9/26/06	Phone questions regarding FDA comments (CTD 900130)
NDA 22-161	9/26/06	Email questions regarding FDA comments (CTD 900130)
NDA 22-161	9/28/06	Phone regarding FDA response to CVT questions regarding sample SDTM submission (CTD 900130)
NDA 22-161	10/13/06	Email regarding FDA response to remaining questions regarding sample SDTM submission (CTD 900130)
NDA 22-161	12/15, 12/18/06	Email request for sample eCTD number
NDA 22-161	2/15/07	Phone regarding copy of 15 Feb 07 Phone-1 from IND 62,862: Request NDA Number
NDA 22-161	2/15/07	Phone regarding copy of 15 Feb 07 Phone-2 from IND 62,862: General User Fee Information
NDA 22-161	3/2/07	Email regarding efficacy dataset specification for FDA Biostatistician for Regadenoson NDA
NDA 22-161	3/5/07	Submission 900162: Sample eCTD Submission
NDA 22-161	3/20-4/2/07	Email of SDTM test submission 900162 and request for feedback on conformity of the submission
NDA 22-161	3/21, 3/23/07	Phone request for SDTM test submission 900162 email to be resent
NDA 22-161	3/21, 4/4/07	Phone regarding sample eCTD submission follow-up recommendation

Submission No.	Date	Content
NDA 22-161	3/29-30/07	Phone regarding follow-up on test submission and feedback from Dr. Mucci on dataset specifications
NDA 22-161	4/3/07	Email regarding follow-up on acceptability of efficacy dataset specification for NDA (as described in email sent on 02 March 2007): EXCEL (or equivalent MINITAB) formatted sets in addition to the SAS formatted sets
NDA 22-161	4/5/07	Phone regarding status of Sample eCTD submission (900162)
NDA 22-161	4/5/07	Phone regarding Sample eCTD submission (900162) processed without any issues
NDA 22-161	4/4-5/07	Email regarding Sample eCTD submission (900162) processed without any issues
NDA 22-161	5/2/07	Phone requesting priority review and post-submission meeting
NDA 22-161	5/2-3/07	Phone regarding NDA submission acknowledgement, Post-submission meeting and Trade Name Pre-clearance
NDA 22-161	5/14/07	Submission of original NDA
NDA 22-161	5/14/07	Email regarding notification that NDA was submitted by CVT today
NDA 22-161	5/18, 5/21/07	Phone regarding Post-submission meeting date and NDA field copy certification
NDA 22-161	5/21/07	Email confirmation of post-submission meeting date
NDA 22-161	5/22/07	Letter acknowledgement of the receipt of NDA dated 14 May 07
NDA 22-161	6/8, 6/11/07	Phone questions regarding NDA 22-161 and Post-submission meeting
NDA 22-161	6/12/07	Email of CVT Attendees to 19 Jun 07 Post-submission meeting
NDA 22-161	6/15/07	Phone regarding details of Post-submission (Applicant Orientation Presentation) Meeting, and Application had been assigned "Standard" review
NDA 22-161	6/15/07	Email of FDA Attendees for the 19 Jun 07 Applicant Orientation Presentation
NDA 22-161	6/17/07	Email of slides for Application Orientation Presentation
NDA 22-161	6/19/07	Minutes-CVT: Meeting between CVT and FDA regarding Applicant Orientation Presentation Meeting
NDA 22-161	6/21/07	Email regarding Applicant Orientation Presentation Follow-up
NDA 22-161	6/21-22/07	Phone regarding location of Information in NDA
NDA 22-161	6/22/07	Email regarding location of Specific Information in NDA 22-161
NDA 22-161	6/26-28/07	Email regarding Society of Nuclear Medicine presentation slides
NDA 22-161	6/27/07	Email list of study investigators
NDA 22-161	6/27/07	Phone request for location of List of Study Investigators
NDA 22-161	7/13-16/07	Phone regarding possible inspections at sites and Imaging Core Lab
NDA 22-161	7/16/07	Email regarding possible inspections at sites and Imaging Core Lab
NDA 22-161	7/18/07	Email of contact information for possible GCP inspections
NDA 22-161	.7/19/07	Email of contact information for possible GCP inspections
NDA 22-161	7/26-30/07	Email of NDA filing letter
NDA 22-161	7/26-31/07	Email regarding CDs for GCP Inspections
NDA 22-161	7/27-30/07	Phone regarding filing letter
NDA 22-161	7/30/07	Fax of NDA filing letter dated 27 Jul 07
NDA 22-161	8/3/07	Letter regarding GCP inspection information sent on CDs by FedEx
NDA 22-161	8/5/07	Email regarding GCP inspection information sent on CDs by FedEx
NDA 22-161	8/7/07	Phone notification of a fax being sent regarding CMC clarification, CMC reviewer's question, and brief discussion on Nonclinical and Clinical questions
NDA 22-161	8/7/07	Fax requesting to provide the correct name, address and CFN number for drug substance manufacturing, in-process testing and release testing site, Hovione LLC

Submission No.	Date	Content
NDA 22-161	8/8, 8/10/07	Email regarding question on how best to provide responses to CMC question
NDA 22-161	8/9/07	Email regarding response to request for drug substance manufacturing site address
NDA 22-161	8/15/07	Phone regarding information from NDA Review Team meeting, brief discussion on CVT's plan for the 4-Month Safety Update, and labeling comments
NDA 22-161	8/17/07	Email regarding response to 27 Jul 07 potential review issues and Nonclinical deficiencies
NDA 22-161	8/22-23/07	Phone regarding inspection at drug substance manufacturer
NDA 22-161	8/27-28/07	Email regarding inquiries from statistician
NDA 22-161	8/30/07	Phone regarding GCP inspections
NDA 22-161	8/30-31/07	Email regarding DIA meeting
NDA 22-161	9/11-14/07	Phone regarding update on Pharm/Tox response and teleconference, questions from statistician, and status of CMC review comments
NDA 22-161	9/11/07	Email of FDA statistician's questions
NDA 22-161	9/14/07	Submission of Safety Update Report and Revised Draft Labeling, and response to 27 Jul 07 filing letter
NDA 22-161	9/15/07	Email regarding confirmation and Dial-in Information for 20 Sep 07 teleconference
NDA 22-161	9/18/07	Email regarding CVT response to FDA statistician's questions
NDA 22-161	9/18/70	Email regarding additional question from FDA statistician
NDA 22-161	9/20/07	Email regarding CVT response to additional FDA statistician's question
NDA 22-161	9/20/07	Minutes-CVT: Potential preclinical review issues from 27 Jul 07 Regadenoson NDA filing
NDA 22-161	9/21-27/07	Phone regarding question on Four-month Safety Update
NDA 22-161	9/24/07	Phone regarding scheduling inspection at core lab
NDA 22-161	9/25-26/07	Email regarding confirmation of GCP inspection dates
NDA 22-161	9/26/07	Email regarding request for feedback on Nonclinical study protocols
NDA 22-161	9/27/07	Email confirming GCP inspections dates
NDA 22-161	10/2/07	Email of letter of confirmation for GCP site inspection
NDA 22-161	10/3/07	Fax regarding Non-clincial study protocols/NDA 22-161 (LEXISCAN™ Regadenoson Injection) and FDA t-con dated 20 Sep 07; and Sponsor response, dated 26 Sep 07
NDA 22-161	10/3-5/07	Phone regarding FDA feedback on Nonclinical protocols, fax of Clinical reviewer comments, and status of CMC reviewer comments
NDA 22-161	10/4/07	Phone regarding dates for additional GCP inspections
NDA 22-161	10/4/07	Email regarding FDA inspections of NDA 22-161
NDA 22-161	10/4-5/07	Phone regarding revised dated for GCP inspections
NDA 22-161	10/5/07	Fax of Clinical comments to Sponsor regarding review of pending NDA
NDA 22-161	10/8/07	Email requesting for clarification of Clinical comments
NDA 22-161	10/9/07	Phone regarding confirmation of inspection dates
NDA 22-161	10/12/07	Phone regarding site inspection
NDA 22-161	10/16/07	Email of CMC IR comments for NDA 22-161 (Regadenoson)
NDA 22-161	10/16/07	Email of information for site inspection
NDA 22-161	10/16/07	Email for plans for travel to inspection sites
NDA 22-161	10/16/07	Email of confirmation letter
NDA 22-161	10/16-17/07	Phone regarding questions on submission of CMC responses, Clinical responses, and Toxicology studies, and no plans for Advisory Committee
NDA 22-161	10/17/07	Email of response to 05 Oct 07 request for additional Clinical information
NDA 22-161	10/17/07	Email of contact information for inspection
NDA 22-161	10/17/07	Phone regarding questions on CMC request for information

Submission No.	Date	Content
NDA 22-161	10/19/07	Submission response to 16 Oct 07 CMC IR comments
NDA 22-161	10/19/07	Email regarding response to 16 Oct 07 CMC IR comments
NDA 22-161	10/19/07	Email regarding travel arrangements
NDA 22-161	10/24-26/07	Phone regarding miscellaneous topics
NDA 22-161	10/25/07	Email regarding changes to Nonclinical protocols and timeline for submission of study reports
NDA 22-161	10/25/07	Email regarding clinical information requested
NDA 22-161	10/26/07	Email of follow-up to clinical questions
NDA 22-161	10/31/07	Email of CVT inspection support team
NDA 22-161	11/1/07	Phone regarding documentation of Quintiles role at Core Lab, question on Low LVEF patients, scheduling demonstration
NDA 22-161	11/1-2/07	Phone regarding submission of stability data, and scheduling "Image Reading" Demonstration
NDA 22-161	11/5/07	Email of CVT contact information
NDA 22-161	11/7/07	Email regarding availability for Image Reading Demonstration, and response to requests for Clinical information
NDA 22-161	11/14/07	Email of Clinical question
NDA 22-161	11/16/07	Letter and Email of CMC information request due by 23 Nov 07
NDA 22-161	11/16/07	Phone regarding timing for response to CMC information request
NDA 22-161	11/26/07	Submission response to 16 Nov 07 CMC information request letter
NDA 22-161	11/26/07	Email of Clinical question
NDA 22-161	11/26-27/07	Email regarding response to 16 Nov 07 CMC IR letter
NDA 22-161	11/29/07	Phone follow-up on submission of CMC and Clinical responses
NDA 22-161	11/30/07	Submission of follow-up response to 16 Oct 07 CMC IR comments and Revised Draft Carton Label for syringe
NDA 22-161	11/30/07	Email of CMC Stability update
NDA 22-161	11/30/07	Submission 26 Nov 07 response to 14 Nov 07 request for Clinical information
NDA 22-161	12/3/07	Submission responses and correspondences related to Nonclinical studies
NDA 22-161	12/3/07	Submission of CVT 5132 clinical study report – correction to AE and SAE listings
NDA 22-161	12/3/07	Email of GCP inspection information
NDA 22-161	12/5/07	Phone regarding update on recent submissions, expecting timing of feedback from DMETS on Tradename, and plans for Image Demonstration at FDA
NDA 22-161	12/12/07	Submission of new Nonclinical study reports CVT3146.149-P, and CVT3146.057-T
NDA 22-161	12/12/07	Letter and Email of CMC Information Request
NDA 22-161	12/14/07	Email of Image Reading Demonstration – 17 Dec 07 List of CVT participants
NDA 22-161	12/17/07	Minutes-CVT: Meeting between CVT and FDA regarding Image Reading Demonstration Meeting Minutes
NDA 22-161	12/18/07	Phone regarding plans for vacations, follow-up after FDA meeting, timing of CMC response, submission of Nonclinical report, and labeling review
NDA 22-161	12/18/07	Submission response to 12 Dec 07 CMC IR letter
NDA 22-161	12/19/07	Email response to 12 Dec 07 CMC IR
NDA 22-161	12/21/07	Submission of new Nonclinical study report of CVT3146.056-T
NDA 22-161	12/21/07	Email regarding submission of final Nonclinical study report
NDA 22-161	12/21/07	Email regarding location of reader segment scores in NDA CRT Datasets
NDA 22-161	1/4/08	Phone regarding information needed for Microbiology Review
NDA 22-161	1/7/08	Submission response to 04 Jan 08 Microbiology Request
NDA 22-161	1/7/08	Email of response to 04 Jan 08 Microbiology Request

Submission No.	Date	Content
NDA 22-161	1/8-11/08	Phone regarding status of feedback on Trade name and NDA Review
NDA 22-161	1/17-18/08	Phone regarding status of Trade name Review and Pharm/Tox Information Request
NDA 22-161	1/22-23/08	Phone regarding status of NDA Review and Trade name Clearance
NDA 22-161	1/22-24/08	Email of question from Pharm/Tox reviewer
NDA 22-161	1/28-29/08	Phone regarding notification of inspection at CVT for CVT 5131 and CVT 5132
NDA 22-161	1/29-30/08	Phone regarding timing/plan for FDA's Labeling comments, and question regarding inspection at CVT
NDA 22-161	1/29-30/08	Phone questions regarding CVT inspection
NDA 22-161	1/31/08	Form FDA 482: FDA inspection of CVT; Announced inspection for Regadenoson (Form FDA 483 inspectional observations was not issued)
NDA 22-161	2/5/08	Phone regarding single dose bridging study results and teleconference with FDA
NDA 22-161	2/7/08	Email of summary document and CVT participants for the teleconference 0 07 Feb 08
NDA 22-161	2/7/08	Minutes-CVT: FDA comments on Nonclinical bridging study results
NDA 22-161	2/11/08	Phone follow-up on Pharm/Tox
NDA 22-161	2/12/08	Phone regarding additional Pharm/Tox discussion
NDA 22-161	2/18/08	Submission of Assessment of clinical relevance of histopathology finding in single dose toxicity study in rats (CVT3146.056-T)
NDA 22-161	2/19/08	Email regarding assessment of histopathology finding from single dose toxicity study (CVT3146.056-T)
NDA 22-161	2/19-20/08	Phone follow-up on Pharm/Tox submission
NDA 22-161	2/20/08	Phone regarding NDA review status
NDA 22-161	2/20/08	Email follow-up on GCP inspection held at CVT for CVT 5131 and CVT 5132
NDA 22-161	2/20/08	Phone regarding response to information request for Nuclear Core Laboratory
NDA 22-161	2/22/08	Letter response to Information Request for Nuclear Core Laboratory
NDA 22-161	2/22/08	Email response to Information Request for Nuclear Core Laboratory (1 of 4)
NDA 22-161	2/22/08	Email response to Information Request for Nuclear Core Laboratory (2 of 4)
NDA 22-161	2/22/08	Email response to Information Request for Nuclear Core Laboratory (3 of 4)
NDA 22-161	2/22/08	Email response to Information Request for Nuclear Core Laboratory (4 of 4)
NDA 22-161	2/25, 2/27/08	Phone regarding follow-up on NDA review
NDA 22-161	2/26/08	Email of CMC and Clinical question
NDA 22-161	2/27/08	Email response to CMC and Clincal question
NDA 22-161	2/27/08	Submission response to 26 Feb 08 Clinical and Chemistry comments
NDA 22-161	2/28/08	Phone regarding outcome of Pharm/Tox assessment and status of NDA review
NDA 22-161	2/28/08	Phone follow-up after teleconference
NDA 22-161	2/28/08	Submission of copy of Summary Document for 07 Feb 08 Pharm/Tox teleconference
NDA 22-161	2/29/08	Phone follow-up on submission of Core Lab documentation
NDA 22-161	3/4/08	Email of Information Request on reading session and reading room
NDA 22-161	3/6/08	Email of response to Information Request on reading session and reading room
NDA 22-161	3/7/08	Email of FDA Draft Label for Regadenoson Injection

Submission No.	Date	Content
NDA 22-161	3/8/08	Email response to question on Access Database
NDA 22-161	3/10/08	Email regarding additional comments on package insert and carton/container labels
NDA 22-161	3/10/08	Email of Regadenoson Labeling
NDA 22-161	3/10-11/08	Phone regarding submission of response to FDA's Draft Labeling
NDA 22-161	3/10-11/08	Phone regarding NDA review status
NDA 22-161	3/11/08	Email regarding adverse reactions email address contact information
NDA 22-161	3/11/08	Email regarding vial/carton package drawings/labeling
NDA 22-161	3/11/08	Email regarding vial/carton package drawings/labeling - REVISED
NDA 22-161	3/11/08	Email regarding comments from CMC and FDA labeling team on CVT package drawings and package insert
NDA 22-161	3/12/08	Email regarding vial/carton package drawings/labeling, additional recommendations on labeling, and question regarding presentation of trade name
NDA 22-161	3/12/08	Phone regarding Trade name and Labeling status
NDA 22-161	3/13/08	Email of Clinical information request
NDA 22-161	3/13/08	Phone regarding label and review status
NDA 22-161	3/13/08	Phone regarding label review
NDA 22-161	3/13/08	Email regarding response to Clinical Information Request
NDA 22-161	3/18/08	Phone regarding labeling status
NDA 22-161	3/20/08	Phone regarding labeling status
NDA 22-161	3/21/08	Phone regarding labeling status
NDA 22-161	3/24/08	Phone regarding labeling/NDA status
NDA 22-161	3/24/08	Email regarding labeling, postmarketing commitments and CVT response to labeling comments
NDA 22-161	3/25/08	Phone regarding NDA status
NDA 22-161	3/25/08	Email regarding CVT participants on 25 March teleconference
NDA 22-161	3/25/08	Minutes-CVT: Meeting minutes for teleconference between CVT, Astellas Pharma US and FDA
NDA 22-161	3/25/08	Minutes-Astellas: Minutes for teleconference between CVT, Astellas Pharma US and FDA
NDA 22-161	3/25/08	Email regarding post-marketing commitments
NDA 22-161	3/25/08	Email regarding revised SPL
NDA 22-161	3/26/08	Phone regarding SPL file and comments on package labeling
NDA 22-161	3/26/08	Email regarding packaging information and CVT response
NDA 22-161	3/27/08	Phone regarding follow-up on package labels, SPL and review
NDA 22-161	3/28/08	Phone regarding status update
NDA 22-161	3/28/08	Email regarding Word version of package drawings
NDA 22-161	3/28/08	Fax: resending post-marketing commitments (25 Mar 2008)
NDA 22-161	4/1-2/08	Phone regarding NDA status
NDA 22-161	4/3/08	Phone regarding NDA status
NDA 22-161	4/4/08	Phone regarding NDA status
NDA 22-161	4/7/08	Phone regarding NDA status
NDA 22-161	4/7/08	Phone regarding NDA status
NDA 22-161	4/8/08	Phone regarding NDA status
NDA 22-161	4/8/08	Email regarding NDA status
NDA 22-161	4/8-9/08	Email regarding NDA status
NDA 22-161	4/9/08	Phone regarding NDA status
NDA 22-161	4/9/08	Email regarding final version of labeling for review
NDA 22-161	4/9/08	Phone regarding NDA status
NDA 22-161	4/9/08	Phone regarding NDA approval

Submission No.	Date	Content
NDA 22-161	4/10/08	Phone regarding NDA status
NDA 22-161	4/10/08	Email: Lexiscan approval letter
NDA 22-161	4/10/08	Phone regarding NDA approval letter
NDA 22-161	4/11/08	Email regarding Lexiscan listing on drugs@fda.com site
NDA 22-161	4/11/08	Phone regarding FDA approval
NDA 22-161	4/11/08	Phone regarding correction of FDA posting of Lexiscan information on drugs@fda.com site

12. Eligibility for extension of patent term

In the opinion of CV Therapeutics, Inc., US Patent No. 6,642,210 is eligible for the requested extension of patent term.

The maximum length of extension available for US Patent No. 6,642,210 based on approval of Lexiscan ™ (regadenoson 0.4 mg/5 mL injectable solution) will be 977 days (2 years, 8 months, and 1 day), and the length of extension will be determined as follows:

The regulatory review period — 35 USC 156(g)(1)(B)

The regulatory review period started on August 2, 2001, the day that IND 62,862 became effective; this was prior to the issuance of US Patent No. 6,642,210 on November 4, 2003. The regulatory review period ended April 10, 2008. The regulatory review period has therefore lasted 6 years, 8 months, and 9 days 2444 days.

As 53 U.S.C. § 156(c) limits the period of time which may be extended to a time "equal to the regulatory review period for the approved product which period occurs after the date the patent is issued," the portion of the regulatory review period which may be used to calculate the extension is limited to 4 years, 5 months, and 6 days which is 1619 days.

The IND period — 35 USC 156(g)(1)(B)(i)

The period from the issuance of US Patent No. 6,642,210 on November 4, 2003, to the date of submission of NDA 22-161 on May 14, 2007, is 3 years, 6 months, 10 days (1287 days); one-half this period is 644 days.

The NDA period — 35 USC 156(g)(1)(B)(ii)

The period from the date of submission of NDA 22-161 on May 14, 2007, to the date of approval of the NDA on April 10, 2008, is 0 years, 10 months, and 28 days (333 days).

Calculation of maximum extension on approval — 35 USC 156(c)

The maximum permissible extension is calculated as the sum of one-half the IND period (644 days) and the whole NDA period (333 days), for a total of at least 977 days (2 years, 8 months, and 1 days). When added to the remaining patent term of U.S. Patent No. 6,642,210, the resulting total term of the patent remaining after the approval of the approved product including the extension is 13 years, 10 months, and 13 days, which does not exceed the maximum 14-year term pursuant to 35 USC 156(c)(3). Accordingly, CV Therapeutics, Inc., expects that the length of extension available for US Patent No. 6,642,210 based on approval of Lexiscan ™ (regadenoson 0.4 mg/5 mL injectable solution), will be 977 days (2 years, 8 months, and 1 day), which will expire on February 23, 2022, when granted.

13. Duty of Disclosure

CV Therapeutics, Inc., acknowledges a duty to disclose to the Director of the US Patent & Trademark Office and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought herein.

In an effort to comply with the aforementioned duty, CV Therapeutics, Inc., wishes to make the Director aware that an Application for Extension of Patent Term for US Patent 6,403,567 has been contemporaneously submitted to the US Patent & Trademark Office on the same day of filing of the present Application.

CV Therapeutics, Inc. also wishes to make the Director aware that CV Therapeutics, Inc. was the NDA applicant throughout the regulatory review period and that the NDA was transferred to Astellas Pharma US, Inc. on April 17, 2008.

14. Fees

The Commissioner is hereby authorized to charge $1,120.00 (37 CFR 1.20(j)(1)) and any underpayment or credit any overpayment to Deposit Account No. 50-1789, referencing matter 99-0423.

15. Name and address for correspondence

Inquiries and correspondence relating to this Application for extension of patent term should be directed to:

Customer Number 27716.

Telephone inquiries should be directed to:

Daniel W. Collins at 650-384-8047
VP, Legal - Intellectual Property
CV Therapeutics, Inc.
3172 Porter Drive
Palo Alto, CA 94304

Faxed correspondence should be directed to:
Daniel W. Collins, 650-475-0359

16. Multiple copies

This Application for extension of patent term is being submitted in an original and two copies. The undersigned hereby certifies that the copies of this Application filed herewith are true and correct copies.

17. Declaration

The undersigned duly authorized agent for CV Therapeutics, Inc., hereby declares that:

(1) he is a patent attorney authorized to practice before the US Patent & Trademark Office and is authorized to represent CV Therapeutics, Inc., in this Application for extension of patent term by virtue of a Power of Attorney executed on June 3, 2008. A copy of the Power of Attorney is attached hereto as Attachment E;

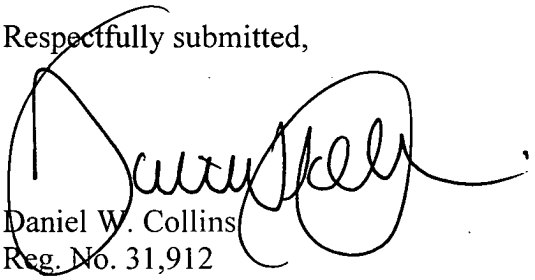
(2) he has reviewed and understands the contents of this Application;

(3) he believes that US Patent No. 6,642,210 is subject to extension pursuant to 35 USC 156(d)(1) and 37 CFR 1.710;

(4) he believes that an extension of the length claimed is justified under 35 USC 156 and the applicable regulations; and

(5) he believes that US Patent No. 6,642,210 meets the conditions for extension of the term of a patent set forth in 37 CFR 1.720.

Respectfully submitted,



Daniel W. Collins
Reg. No. 31,912
VP, Legal - Intellectual Property
CV Therapeutics, Inc.

June 6, 2008

Customer No. 27716
CV Therapeutics, Inc.
3172 Porter Drive
Palo Alto, CA 94304
Telephone: 650-384-8047
Facsimile: 650-475-0359
daniel.collins@cvt.com

This is to certify that the copy of this Application (together with the appended Attachments "A" through "E" filed herewith is a true and correct duplicate.

Date: June 6, 2008

Daniel W. Collins

ATTACHMENT A
COPY OF US PATENT NO. 6,642,210

US006642210B1

(12) **United States Patent**
Zablocki et al.

(10) **Patent No.: US 6,642,210 B1**
(45) **Date of Patent: *Nov. 4, 2003**

(54) **2-(N-PYRAZOLO)ADENOSINES WITH APPLICATION AS ADENOSINE A_{2A} RECEPTOR AGONISTS**

(75) Inventors: **Jeff A. Zablocki**, Mountain View, CA (US); **Elfatih O. Elzein**, Freemont, CA (US); **Venkata P. Palle**, Sunnyvale, CA (US)

(73) Assignee: **CV Therapeutics, Inc.**, Palo Alto, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **10/018,446**

(22) PCT Filed: **Jun. 21, 2000**

(86) PCT No.: **PCT/US00/40281**

§ 371 (c)(1),
(2), (4) Date: **Apr. 12, 2000**

(87) PCT Pub. No.: **WO00/78779**

PCT Pub. Date: **Dec. 28, 2000**

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/338,185, filed on Jun. 22, 1999, now Pat. No. 6,403,567.

(51) **Int. Cl.**[7] **A61K 31/70**; C07H 19/20; C07H 19/67

(52) **U.S. Cl.** **514/46**; 536/226.3; 536/26.6; 536/27.61; 536/27.62; 536/27.63

(58) **Field of Search** 514/46, 47; 536/26.3, 536/26.6, 27.61, 27.62, 27.63

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,956,345	A	9/1990	Miyasaka et al.	514/46
4,968,697	A *	11/1990	Hutchison	514/46
5,070,877	A	12/1991	Mohiuddin et al.	
5,189,027	A	2/1993	Miyashita et al.	514/46
5,270,304	A	12/1993	Kogi et al.	514/46
5,459,254	A	10/1995	Yamaguchi et al.	536/27.11
5,593,975	A	1/1997	Cristalli	514/46
5,705,491	A	1/1998	Yamada	514/46
5,770,716	A	6/1998	Khan et al.	536/23.1
5,939,543	A	8/1999	Morozumi et al.	536/27.63
6,403,567	B1 *	6/2002	Zablocki et al.	514/46

FOREIGN PATENT DOCUMENTS

CA	965411		4/1975
EP	0 354 638	A2 *	2/1990
JP	Hei 5-9197		1/1993

OTHER PUBLICATIONS

Marumoto, et al., "Synthesis and Coronary Vasodilating Activity of 2–Substituted Adenosines", *Chem.. Pharm. Bull.* 23(4): 759–774 (1975).

Marumoto, et al., "Synthesis and Enzymatic Activity of Adenosine 3', 5'–Cyclic Phosphate Analogs", *Chem.. Pharm. Bull.* 27(4) 990–1003 (1979).

Persson, et al., "Synthesis and Antiviral Effects of 2–Heteroaryl Substituted Adenosine and 8–Heteroaryl Substituted Adenosine Guanosine Derivatives", *Bioorganic & Medicinal Chemistry*, 3:1377–1382 (1995).

Mager, et al., "Molecular simulation applied to 2–(N'alkylidenehydrazino)–and 2–(N'aralkylidenehydrazino) adenosine A_2 Agnonists", *Eur J. Med. Chem*, 30:15–25 (1995).

* cited by examiner

Primary Examiner—James O. Wilson
Assistant Examiner—Lawrence E. Crane
(74) *Attorney, Agent, or Firm*—McDonnell Boehnen Hulbert & Berghoff

(57) **ABSTRACT**

N-pyrazole substituted 2-adenosine compounds and methods for using the compounds as A_2A-adenosine receptor agonists useful to stimulate mammalian coronary vasodilation for therapeutic purposes and as adjuncts in cardiological imaging.

18 Claims, 3 Drawing Sheets

FIG. 1A

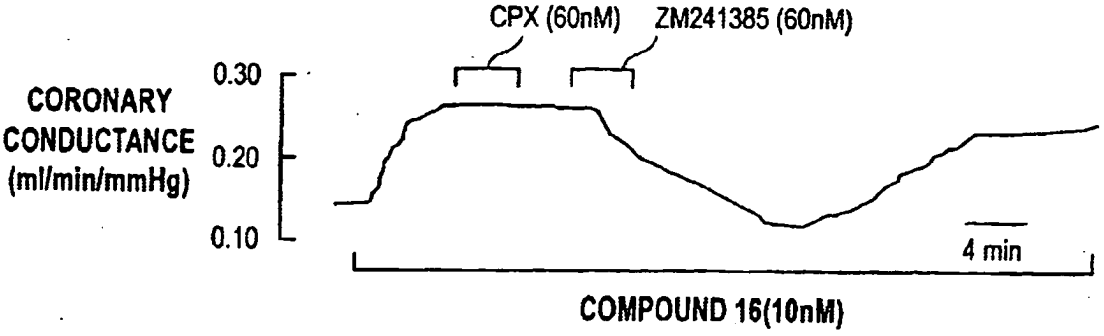

CPX (60nM)
ZM241385 (60nM)
CORONARY CONDUCTANCE (ml/min/mmHg)
0.30
0.20
0.10
4 min
COMPOUND 16(10nM)

FIG. 1B

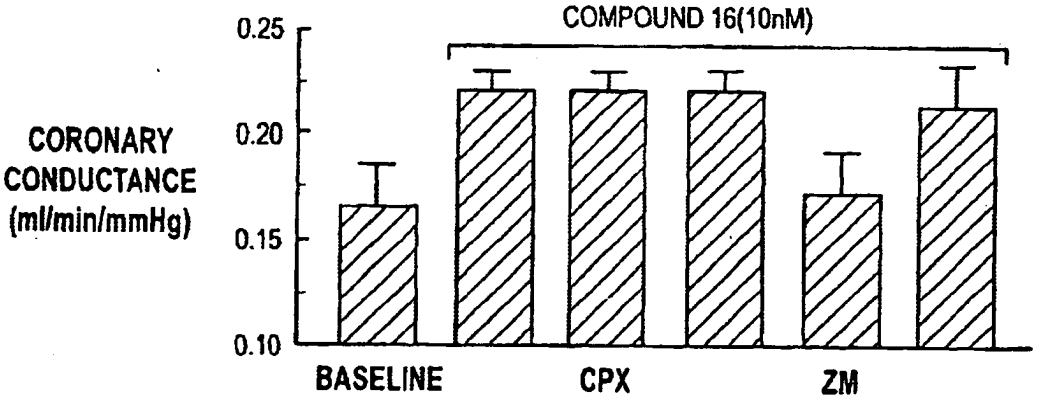

COMPOUND 16(10nM)
CORONARY CONDUCTANCE (ml/min/mmHg)
0.25
0.20
0.15
0.10
BASELINE
CPX
ZM

FIG. 2

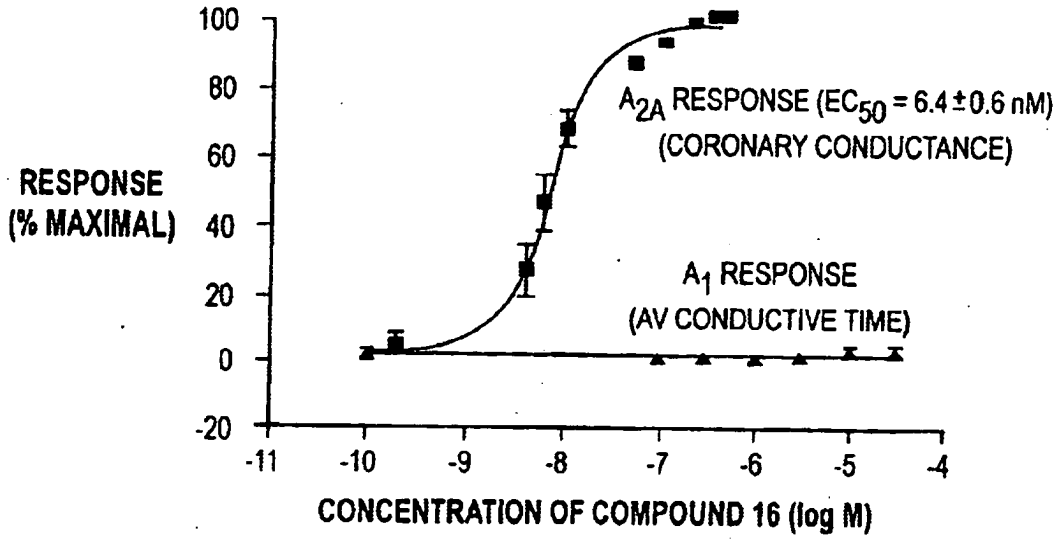

RESPONSE
(% MAXIMAL)
100
80
60
40
20
0
-20
-11
-10
-9
-8
-7
-6
-5
-4
A2A RESPONSE (EC50 = 6.4 ± 0.6 nM)
(CORONARY CONDUCTANCE)
A1 RESPONSE
(AV CONDUCTIVE TIME)
CONCENTRATION OF COMPOUND 16 (log M)

FIG. 3

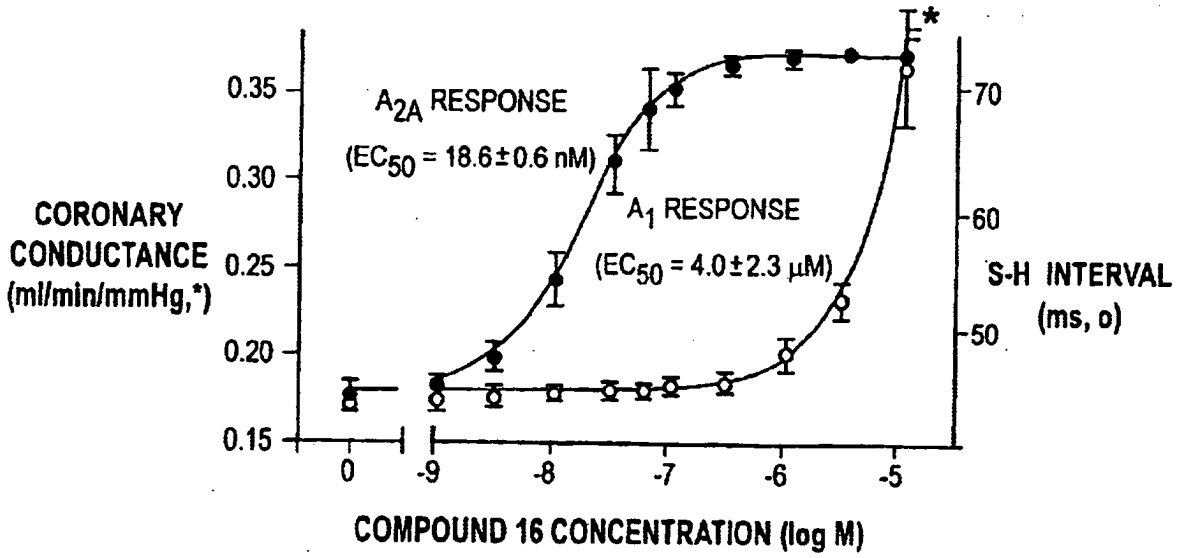
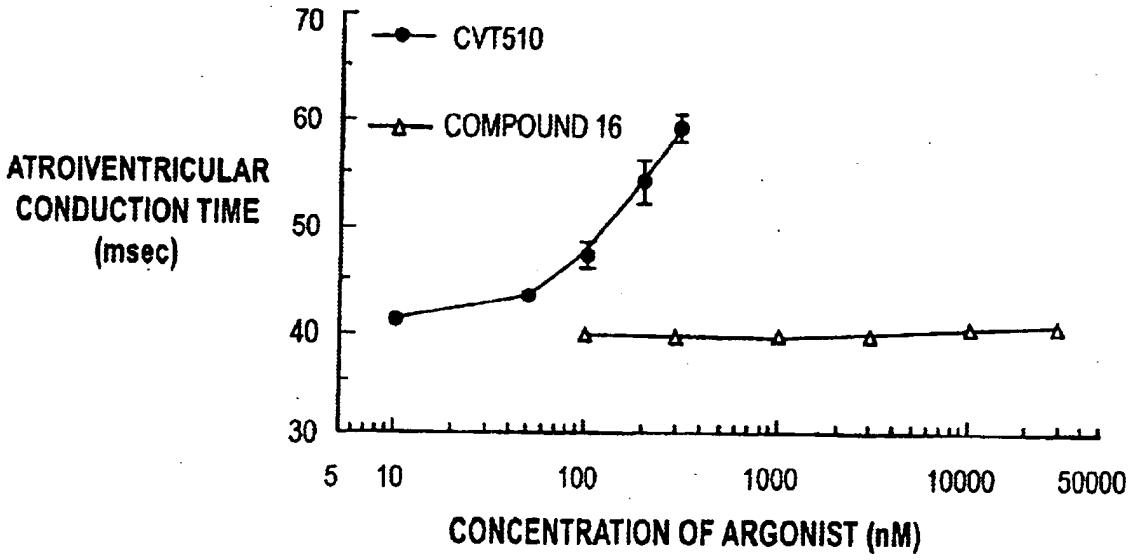

CORONARY
CONDUCTANCE
(ml/min/mmHg,*)
0.35
0.30
0.25
0.20
0.15
0
-9
-8
-7
-6
-5
70
60
50
S-H INTERVAL
(ms, o)
*
A2A RESPONSE
(EC50 = 18.6 ± 0.6 nM)
A1 RESPONSE
(EC50 = 4.0 ± 2.3 μM)
COMPOUND 16 CONCENTRATION (log M)

FIG. 4


70
60
50
40
30
CVT510
COMPOUND 16
ATROIVENTRICULAR CONDUCTION TIME (msec)
5
10
100
1000
10000
50000
CONCENTRATION OF ARGONIST (nM)

2-(N-PYRAZOLO)ADENOSINES WITH APPLICATION AS ADENOSINE A_{2A} RECEPTOR AGONISTS

This application is a 371 of PCT/US00/40281 filed Jun 21, 2000 which is a continuation in part of Ser. No. 09/338,185, filed Jun. 22, 1999 now U.S. Pat. No. 6,403,567.

BACKGROUND OF THE INVENTION

1. Field of Invention

This invention includes N-pyrazole substituted 2-adenosine compounds that are useful as A_{2A} receptor agonists. The compounds of this invention are vasodilating agents that are useful as heart imaging aids that aid in the identification of mammals, and especially humans who are suffering from coronary disorders such poor coronary perfusion which is indicative of coronary artery disease (CAD). The compounds of this invention can also be used as therapeutics for coronary artery disease as well as any other disorders mediated by the A_{2A} receptor.

2. Description of the Art

Pharmacological stress is frequently induced with adenosine or dipyridamole in patients with suspected CAD before imaging with T1 scintigraphy or echocardiography. Both drugs effect dilation of the coronary resistance vessels by activation of cell surface A_2 receptors. Although pharmacological stress was originally introduced as a mean of provoking coronary dilation in patients unable to exercise, several studies have shown that the prognostic value of ^{201}T1 or echocardiographic imaging in patients subjected to pharmacological stress with adenosine or dipyridamole was equivalent to patients subjected to traditional exercise stress tests. However, there is a high incidence of drug-related adverse side effects during pharmacological stress imaging with these drugs such as headache and nausea, that could be improved with new therapeutic agents.

Adenosine A_{2B} and A3 receptors are involved in a mast cell degranulation and, therefore, asthmatics are not give the non-specific adenosine agonists to induce a pharmacological stress test. Additionally, adenosine stimulation of the A_1 receptor in the atrium and A-V node will diminish the S-H interval which can induce AV block (N. C. Gupto et al.; *J. Am Coll. Cardiol*; (1992) 19: 248–257). Also, stimulation of the adenosine A_1 receptor by adenosine may be responsible for the nausea since the Al receptor is found in the intestinal tract (J. Nicholls et al.; *Eur. J. Pharm.* (1997) 338(2) 143–150).

Animal data suggests that specific adenosine A_{2A} subtype receptors on coronary resistance vessels mediate the coronary dilatory responses to adenosine, whereas subtype A_{2B} receptor stimulation relaxes peripheral vessels (note: the latter lowers systemic blood pressure). As a result there is a need for pharmaceutical compositions that are A_{2A} receptor agonists that have no pharmacological effect as a result of stimulating the A_1 receptor in vivo. Furthermore, there is a need for A_{2A} receptor agonists that have a short half-life, and that are well tolerated by patients undergoing pharmacological coronary stress evaluations.

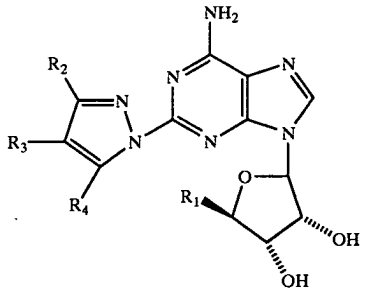
SUMMARY OF THE INVENTION

In one aspect, this invention includes 2-adenosine N-pyrazole compounds that are useful A_{2A} receptor agonists.

In another aspect, this invention includes pharmaceutical compounds including 2-adenosine N-pyrazole that are well tolerated with few side effects.

Still another aspect of this invention are N-pyrazole compounds that can be easily used in conjunction with radioactive imaging agents to facilitate coronary imaging.

In one embodiment, this invention includes 2-adenosine N-pyrazole compounds having the following formula:



In another embodiment, this invention includes methods for using compounds of this invention to stimulate coronary vasodilatation in mammals, and especially in humans, for stressing the heart induced steal situation for purposes of imaging the heart

In still another embodiment, this invention is a pharmaceutical composition comprising one or more compounds of this invention and one or more pharmaceutical excipients.

DESCRIPTION OF THE FIGURES

FIG 1A is a analog record of the increase in coronary conductance caused by Compound 16 of this invention before and after infusions of CPX and ZM241385;

FIG. 1B is a summary of the data shown in FIG. 1A showing that CPX did not but that ZM241385 did attenuate the increase in coronary conductance caused by Compound 16 of this invention. In FIG. 1B, the bars represent mean±SEM of single measurement from 6 rat isolated perfused hearts;

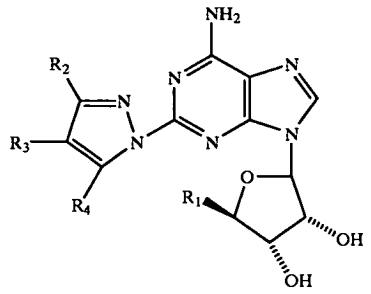
FIG. 2 is a concentration response curve for the A_1 adenosine receptor (AdoR)-mediated negative dromotropic (AV conduction time) and A_{2A} AdoR-mediated vasodialator (increase coronary conductance) effects of Compound 16 in rat isolated perfused hearts. Symbols and error bars indicate means±SEM of single determination from each of four hearts. EC_{50} value (potency) is the concentration of Compound 16 that causes 50% of maximal response;

FIG. 3 is a concentration response curve for the Al adenosine receptor (AdoR)-mediated negative dromotropic (AV conduction time) and A_{2A} AdoR-mediated vasodialator (increase coronary conductance) effects of Compound 16 in guinea pig isolated perfused hearts. Symbols and error bars indicate means±SEM of single determination from each of four hearts. EC_{50} value (potency) is the concentration of Compound 16 that causes 50% of maximal response; and

FIG. 4 is a plot of the effect of CVT510, an A_1 adenosine receptor agonist and Compound 16 of this invention, an A_{2A} adenosine receptor agonist on atrioventricular (AV) conduction time in rat isolated perfused hearts.

DESCRIPTION OF THE CURRENT EMBODIMENT

This invention includes a class of 2-adenosine N-pyrazole having the formula:



wherein R^1=CH_2OH, —$CONR_5R_6$;

R^3 is independently selected from the group consisting of C_{1-15} alkyl, halo, NO_2, CF_3, CN, OR^{20}, SR^{20}, $N(R^{20})_2$, $S(O)R^{22}$, SO_2R^{22}, $SO_2N(R^{20})_2$, $SO_2NR^{20}COR^{22}$, $SO_2NR^{20}CO_2R_{22}$, $SO_2NR^{20}CON(R^{20})_2$, $N(R^{20})_2$ $NR^{20}COR^{22}$, $NR^{20}CO_2R^{22}$, $NR^{20}CON(R^{20})_2$, $NR^{20}C(NR^{20})NHR^{23}$, COR^{20}, CO_2R^{20}, $CON(R^{20})_2$, $COR^{20}SO_2R^{22}$, $NR^{20}SO_2R^{22}$, $SO_2NR^{20}CO_2R^{22}$, $OCONR^{20}SO_2R^{22}$, $OC(O)R^{20}$, $C(O)OCH_2OC(O)R^{20}$, and $OCON(R^{20})_2$,—$CONR^7R^8$, C_{2-15} alkenyl, C_{2-15} alkynyl, heterocyclyl, aryl, and heteroaryl, wherein the alkyl, alkenyl, alkynyl, aryl, heterocyclyl and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, alkyl, NO_2, heterocyclyl, aryl, heteroaryl, CF_3, CN, OR^{20}, SR^{20}, $N(R^{20})_2$, $S(O)R^{22}$, SO_2R^{22}, $SO_2N(R^{20})_2$, $SO_2NR^{20}COR^{22}$, $SO_2NR^{20}CO_2R^{22}$, $SO_2NR^{20}CON(R^{20})_2$, $N(R_{20})_2$ $NR^{20}COR^{22}$, $NR^{20}CO_2^{22}$, $NR^{20}CON(R_{20})_2$, $NR^{20}C(NR^{20})NHR^{23}$, COR^{20}, CO_2R^{20}, $CON(R^{20})_2$, $CONR^{20}SO_2R^{22}$, $NR^{20}SO_2R^{22}$, $SO_2NR^{20}COR^{22}$, $OCONR^{20}SO_2R^{22}$, $OC(O)R^{20}$, $C(O)OCH_2OC(O)R^{20}$, and $OCON(R^{20})_2$ and wherein the optional substituted heteroaryl, aryl, and heterocyclyl substituents are optionally substituted with halo, NO_2, alkyl, CF3, amino, mono- or di- alkylamino, alkyl or aryl or heteroaryl amide, $NCOR^{22}$, $NR^{20}SO_2R^{22}$, COR^{20}, CO_2R^{20}, $CON(R^{20})_2$, $NR^{20}CON(R^{20})_2$, $OC(O)R^{20}$, $OC(O)N(R^{20})_2$, SR^{20}, $S(O)R^{22}$, SO_2R^{22}, $SO_2N(R^{20})_2$, CN, or OR^{20};

R^5 and R^6 are each individually selected from H, and C_1–C_{15} alkyl that is optionally substituted with from 1 to 2 substituents independently selected from the group of halo, NO_2, heterocyclyl, aryl, heteroaryl, CF_3, CN, OR^{20}, SR^{20}, $N(R^{20})_2$, $S(O)R^{22}$, SO_2R^{22}, $SO_2N(R^{20})_2$, SO_2NR^{20}, COR^{22}, $SO_2NR^{20}C_2R^{22}$, $SO_2NR^{20}CON(R^{20})_2$, $N(R^{20})_2$, $NR^{20}COR^{22}$, $NR^{20}CO_2R^{22}$, $NR^{20}CON(R^2)_2$, $NR^{20}C(NR^{20})NHR^{23}$, COR^{20}, CO_2R^{20}, $CON(R^{20})_2$, $CONR^{20}SO_2R^{22}$, $NR^{20}SO_2R^{22}$, $SO_2NR^{20}CO_2R^{22}$, $OCONR^{20}SO_2R^{22}$, $OC(O)R^{20}$, $C(O)OCH_2OC(O)R^{20}$, and $OCON(R^2)_2$ wherein each optional substituted heteroaryl, aryl, and heterocyclyl substituent is optionally substituted with halo, NO_2, alkyl, CF_3, amino, monoalkylamino, dialkylamino, alkylarnide, arylamide, heteroarylamide, $NCOR^{22}$, $NR^{20}SO_2R^{22}$, COR^{20}, CO^2R^{20}, $CON(R^{20})_2$, $NR^{20}CON(R^{20})_2$, $OC(O)R^{20}$, $OC(O)N(R^{20})_2$, SR^{20}, $S(O)R^{22}$, SO_2R^{22}, $SO_2N(R^{20})_2$, CN, and OR^{20};

R^7 is selected from the group consisting of hydrogen, C_{1-15} alkyl, C_{2-15} alkenyl, C_{2-15} alkynyl, heterocyclyl, aryl and heteroaryl, wherein the alkyl alkenyl, alkynyl, aryl, heterocyclyl and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group of halo, NO_2, heterocyclyl aryl, heteroaryl, CF_3, CN, OR^{20}, SR^{20}, $N(R^{20})_2$ $S(O)R^{22}$, SO_2R^{22}, $SO_2N(R^{20})_2$, $SO_2NR^{20}COR^{22}$, $SO_2NR^{20}CO_2R^{22}$, $SO_2NR^{20}CON(R^{20})_2$, $N(R20)_2$ $NR^{20}COR^{22}$, $NR^{20}C_2R^{22}$, $NR^{20}CON(R^{20})_2$, $NR^{20}C(NR^{20})NHR^{23}$, COR^{20}, CO_2R^{20}, $^{CON(R20)}{}_2$, $CONR^{20}SO_2R^{22}$, $NR^{20}SO_2R^{22}$, $SO_2NR^{20}CO_2R^{22}$, $OCONR^{20}SO_2R^{22}$, $OC(O)R^{20}$, $C(O)OCH_2OC(O)R^{20}$ and $OCON(R^{20})_2$ and wherein each optional substituted heteroaryl, aryl and heterocyclyl substituent is optionally substituted with halo, NO_2, alkyl, CF_3, amino, mono- or di- alkylamino, alkyl or aryl or heteroaryl amide, $NCOR^{22}$, $NR^{20}SO_2R^{22}$, COR^{20}, CO_2R^{20}, $CON(R^{20})_2$, $NR^{20}CON(R^{20})_2$, $OC(O)R^{20}$, $OC(O)N(R^{20})_2$, SR^{20}, $S(O)R^{22}$, SO_2R^{22}, $SO_2N(R^{20})_2$, CN, and OR^{20};

R^8 is selected from the group consisting of hydrogen, C_{1-15} alkyl, C_{2-15} alkenyl, C_{2-15} alkynyl, heterocyclyl, aryl, and heteroaryl wherein the alkyl alkenyl, alkynyl, aryl heterocyclyl, and hetemaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, NO_2, heterocyclyl, aryl, heteroaryl, CF_3, CN, OR^{20}, SR^{20}, $N(R^2)_2$, $S(O)R^{22}$, SO_2R^{22}, $SO_2N(R^{20})_2$, $SO_2NR^{20}COR_{22}$, $SO_2NR^{20}CO_2R^{22}SO_2NR^{20}CON(R_{20})_2$, $N(R^{20})_2$ $NR_{20}COR^{22}$, $NR^{20}CO_2R^{22}$, $NR^{20}CON_{20}(R^{20})_2$, $SO_2NR^{20}CO_2R^{22}$, $OCONR^{20}SO_2R^{22}$, $OC(O)R^{20}$, $C(O)OCH_2OC(O)R^{20}$ and $OCON(R^{20})_2$ and wherein each optional substituted heteroaryl, aryl, and heterocyclyl substituent is optionally substituted with halo, NO_2, alkyl, CF_3, amino, mono- or di-alkylamino, alkyl or aryl or heteroaryl amide, $NCOR^{22}$, $NR^{20}SO_2R^2$, COR^{20}, CO_2R^{20}, $CON(R^{20}{}_{20}$, NR^{20} $CON(R^{20}CON(R^{20})_2$, $OC(O)R^{20}$, $OC(O)N(R^2)_2$, SR^{20}, $S(O)R^{22}$, SO_2R^{22}, $SO_2N(R^{20})_2$, CN, and OR^{20};

R^8 is selected from the group consisting of H, C_{1-15} alkyl, C_{2-15} alkenyl, C_{2-15} alkynyl, heterocyclyl, aryl, and heteroaryl, wherein the alkyl alkenyl, alkynyl, heterocyclyl, aryl, and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, NO_2, heterocyclyl, aryl, heteroyl, CF_3, CN, OR^{20}, $N(R^{20})_2$, $S(O)R^{22}$, SO_2R^{22}, $SO_2N(R^{20})_2$, $SO_2NR^{20}COR^{22}$, $SO_2NR^{20}CO_2R^{22}$, $SO_2NR^{20}CON(R^{20})_2$, $N(R^{20})_2$ $NR^{20}COR^{22}$, $NR^{20}CO_2R^{22}$, $NR^{20}CON(R^{20})_2$, $NR^{20}C(NR^{20})NHR^{23}$, COR^{290}, COR^{20}, $CON(R^{20})_2$, $CONR^{20}SO_2R^{22}$, $NR^{20}SO_2R^{22}$, $SO_2NR^{20}CO_2R^{22}$, $OCONR^{20}SO_2R^{22}$, $OC(O)R^{20}$, $C(O)OCH_2OC(O)R^{20}$, and $OCON(R^{20})_2$ and wherein each optional susbtituted hetereoaryl, aryl and heterocyclyl sustitutet is optionally sustitutd with halo, NO^2, alkyl, CF_3, amino- or di-alkylamino, alkyl or aryl or heteroaryl amide, $NCOR^{22}$, $NR^{20}SO_2R^{22}$, COR^{20}, CO_2R^{20}, $CON(R^{20})_2NR^{20}CON(R^{20})_2$, $OC(O)R^{20}$, $OC(O)N(R^{20})_2$, SR^{20}, $S(O)R^{22}$, SO_2R^{22}, $SO_2N(R^{20}{}_2$, CN, and OR^{20};

R^{20} is selected from the group consisting of H, C_{1-15} alkenyl, C_{2-15} alkynyl, heterocyclyl, aryl, and

heteroaryl, wherein the alkyl, alkenyl, alkynyl, heterocyclyl, aryl, and heteroaryl sustituents are optionally substituted with from 1 to 3 substituents independently selected from halo, alkyl, mono- or dialkylamino, alkyl or aryl or heteroaryl amide, CN, O—C_{1-6}, alkyl, CF_3, aryl, and heteroaryl;

R^{22} is selected from the group consisting of C_{1-15} alkyl, C_{2-15} alkenyl, C_{2-15} alkynyl, heterocyclyl, aryl, and heteroaryl, wherein the alkyl, alkenyl, alkynyl, heterocyclyl, aryl, and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from halo, alkyl mono- or dialkylamino, alkyl or aryl or heteroaryl amide, CN, O—C_{1-6} alkyl, CF_3, aryl, and heteroaryl; and

wherein R^2 and R^4 are selected from the group consisting of H. $C_{1-15\ 6}$ alkyl and aryl, wherein the alkyl and aryl substituents are optionally substituted with halo, CN, CF_3, OR^{20} and $N(R^{20})_2$with the proviso that when R^2 is not hydrogen then R^4 is hydrogen, and when R^4 is not hydrogen then R^2 is hydrogen.

In preferred compounds of this invention, R^3 is selected from the group consisting of C_{1-15} alkyl, halo, CF_3, CN, OR^{20}, SR^{20}, $S(O)R^{22}$, SO_2R^{22}, $SO_2N(R^{22})_2$, COR^{20}, CO_2R^{20}, —$CONR^7R^8$, aryl and heteroaryl wherein the alkyl, aryl and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, aryl, heteroaryl, CF3, CN, OR^{20}, SR^{20}, $S(O)R^{22}$, SO_2R^{22}, $SO_2N(R^{20})_2$, COR^{20}, CO_2R^{20} or $CON(Rn_{20})_2$, and each optional heteroaryl and aryl substituent is optionally substituted with halo, alkyl, CF_3 CN, and OR^{20}; R^5 and R^6 are independently selected from the group of H and C_{1-15} alkyl including one optional aryl substituent and each optional aryl substituent that is optionally substituted with halo or CF3; R7 is selected from the group consisting of C_{1-15} alkyl, C_{2-15} alkynyl, aryl, and heteroaryl, wherein the alkyl, alkynyl aryl, and heteroaryl substituents are optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, aryl, heteroaryl, CF_3, CN, OR^{20}, and each optional heteroaryl and aryl substituent is optionally substituted with halo, alkyl, CF_3 CN, or OR^{20}; R^8 is selected from the group consisting of hydrogen and C_{1-15} alkyl; R^{20} is selected from the group consisting of H, C_{1-4}, alkyl and aryl, wherein alkyl and aryl substituents are optionally substituted with one alkyl substituent; and R^{22} is selected from the group consisting of H, C_{1-4} alkyl and aryl which are each optionally substituted with from 1 to 3 alkyl group.

In more preferred compounds, R^1 is CH_2OH; R^3 is selected from the group consisting of CO_2R^{20}, —$CONR^7R^8$ and aryl where the aryl substituent is optionally substituted with from 1 to 2 substituents independently selected from the group consisting of halo, C_{1-4} alkyl, CF_3 and OR^{20}; R^7 is selected from the group consisting of hydrogen, C_{1-8} alkyl and aryl, where the alkyl and aryl substituents are optionally substituted with one substituent selected from the group consisting of halo, aryl, CF_3, CN, O R^{20} and wherein each optional aryl substituent is optionally substituted with halo, alkyl, CF_3 CN, and OR^{20}; R^8 is selected from the group consisting of hydrogen and C_{1-8} alkyl; and R^{20} is selected from hydrogen and C_{1-4} alkyl.

In a still more preferred embodiment, R^1=CH_2OH; R^3 is selected from the group consisting of CO_2R^{20}, —$CONR^7R^8$, and aryl that is optionally substituted with one substituent selected from the group consisting of halo, C_{1-3} alkyl and OR^{20}; R^7 is selected from of hydrogen, and C^{1-3} alkyl; R^8 is hydrogen; and R^{20} is selected from hydrogen and C_{1-4} alkyl. In this preferred embodiment, R^3 is most preferably selected from —CO_2Et and —CONHEt.

In another still more preferred embodiment, R^1=—CONHEt, R^3 is selected from the group consisting of CO_2R^{20}, —$CONR^7R^8$, and aryl in that aryl is optionally substituted with from 1 to 2 substituents independently selected from the group consisting of halo, C_{1-3} alkyl, CF_3 or OR^{20}; R^7 is selected from the group consisting of hydrogen, and C_{1-8} alkyl that is optionally substituted with one substituent selected from the group consisting of halo, CF_3, CN or OR^{20}; R^8 is selected from the group consisting of hydrogen and C^{1-3} alkyl; and R^{20} is selected from the group consisting of hydrogen and C^{1-4} alkyl. In this more preferred embodiment, R^8 is preferably hydrogen, R^7 is preferably selected from the group consisting of hydrogen, and C^{1-3}, and R^{20} is preferably selected from the group consisting of hydrogen and C_{1-4} alkyl.

In a most preferred embodiment, the compound of this invention is selected from ethyl1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylate, (4S,2R,3R,5R)-2-{6-amino-2-[4(-4-chlorophenyl pyrazolyl]purin-9-yl}-5-(hydroxymethyl) oxolane-3,4-diol, (4S,2R,3R,5R)-2-{6-amino-2-[4(4-methoxyphenyl)pyrazolyl]purin-9-yl}-5-(hydroxymethyl) oxolane-3,4-diol, (4S,2R,3R,5R)-2-{6-amino-2-[4-(4-methylphenyl)pyrazolyl]purin-9-yl}-5-(hydroxymethyl) oxolane-3,4-diol, (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide, 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4carboxylic acid, (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N,N-dimethylcarboxamide, (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-ethylcarboxamide, 1-{9-[(4S,2R,3R,5R-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxamide, 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-(cyclopentylmethyl) carboxamide, (1-{9-[(4S,2R, 3R,5R)-3,4-dihydroxy-5-hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-[(4-chlorophenyl)methyl]carboxamide, Ethyl 2-[(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl) carbonylamino]acetate, and mixtures thereof.

The following definitions apply to terms as used herein.

"Halo" or "Halogen"—alone or in combination means all halogens, that is, chloro (Cl), fluoro (F), bromo (Br), iodo (I).

"Hydroxyl" refers to the group —OH.

"Thiol" or "mercapto" refers to the group —SH.

"Alkyl"—alone or in combination means an alkane-derived radical containing from 1 to 20, preferably 1 to 15, carbon atoms (unless specifically de fined). It is a straight chain alkyl, branched alkyl or cycloalkyl. Preferably, straight or branched alkyl groups containing from 1–15, more preferably 1 to 8, even more preferably 1–6, yet more preferably 1–4 and most preferably 1–2, carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, t-butyl and the like. The term "lower alkyl" is used herein to describe the straight chain alkyl groups described immediately above. Preferably, cycloalkyl groups are monocyclic, bicyclic or tricyclic ring systems of 3–8, more preferably 3–6, ring members per ring, such as cyclopropyl, cyclopentyl, cyclohexyl, adamantyl and the like. Alkyl also includes a straight chain or branched alkyl group that contains or is interrupted by a cycloallyl portion. The straight chain or branched alkyl group is attached at any available point to produce a stable compound. Examples of this include, but

are not limited to, 4-(isopropyl)-cyclohexylethyl or 2-methyl-cyclopropylpentyl. A substituted alkyl is a straight chain alkyl, branched alkyl, or cycloalkyl group defined previously, independently substituted with 1 to 3 groups or substituents of halo, hydroxy, alkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, acyloxy, aryloxy, heteroaryloxy, amino optionally mono- or di-substituted with alkyl, aryl or heteroaryl groups, amidino, urea optionally substituted with alkyl, aryl, heteroaryl or heterocyclyl groups, aminosulfonyl optionally N-mono- or N,N-di-substituted with alkyl, aryl or heteroaryl groups, alkylsulfonylamino, arylsulfonylamino, heteroarylsulfonylamino, alkylcarbonylamino, arylcarbonylamino, heteroarylcarbonylamino, or the like.

"Alkenyl"—lone or in combination means a straight, branched, or cyclic hydrocarbon containing 2–20, preferably 2–17, more preferably 2–10, even more preferably 2–8, most preferably 2–4, carbon atoms and at least one, preferably 1–3, more preferably 1–2, most preferably one, carbon to carbon double bond. In the case of a cycloalkyl group, conjugation of more than one carbon to carbon double bond is not such as to confer aromaticity to the ring. Carbon to carbon double bonds may be either contained within a cycloalkyl portion, with the exception of cyclopropyl, or within a straight chain or branched portion. Examples of alkenyl groups include ethenyl, propenyl, isopropenyl, butenyl, cyclohexenyl, cyclohexenylalkyl and the like. A substituted alkenyl is the straight chain alkenyl branched alkenyl or cycloalkenyl group defined previously, independently substituted with 1 to 3 groups or substituents of halo, hydroxy, alkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, acyloxy, aryloxy, heteroaryloxy, amino optionally mono- or di-substituted with alkyl, aryl or heteroaryl groups, amidino, urea optionally substituted with alkyl, aryl, heteroaryl or heterocyclyl groups, aminosulfonyl optionally N-mono- or N,N-di-substituted with alkyl, aryl or heteroaryl groups, alkylsulfonylamino, arylsulfonylamino, heteroarylsulfonylamino, alkylcarbonylamino, arylcarbonylamino, heteroarylcarbonylamino, carboxy, alkoxycarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, or the like attached at any available point to produce a stable compound.

"Alkynyl"—lone or in combination means a straight or branched hydrocarbon containing 2–20, preferably 2–17, more preferably 2–10, even more preferably 2–8, most preferably 2–4, carbon atoms containing at least one, preferably one, carbon to carbon triple bond. Examples of alkynyl groups include ethynyl, propynyl, butynyl and the like. A substituted alkynyl refers to the straight chain alkynyl or branched alkenyl defined previously, independently substituted with 1 to 3 groups or substituents of halo, hydroxy, alkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, acyloxy, aryloxy, heteroaryloxy, amino optionally mono- or di-substituted with alkyl, aryl or heteroaryl groups, amidino, urea optionally substituted with alkyl, aryl, heteroaryl or heterocyclyl groups, aminosulfonyl optionally N-mono- or N,N-di-substituted with alkyl, aryl or heteroaryl groups, alkylsulfonylamino, arylsulfonylamino, heteroarylsulfonylamino, alkylcarbonylamino, arylcarbonylamino, heteroarylcabonylamino, or the like attached at any available point to produce a stable compound.

"Alkyl alkenyl" refers to a group —R—CR'=CR'" R"", where R is lower alkyl, or substituted lower alkyl, R', R'", R"" may independently be hydrogen, halogen, lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, hetaryl, or substituted hetaryl as defined below.

"Alkyl alkynyl" refers to a groups —RC=CR' where R is lower alkyl or substituted lower alkyl, R' is hydrogen, lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, hetaryl, or substituted hetaryl as defined below.

"Alkoxy" denotes the group —OR, where R is lower alkyl, substituted lower alkyl, acyl, aryl, substituted aryl, aralkyl, substituted aralkyl, heteroalkyl, heteroarylalkyl, cycloalkyl, substituted cycloalkyl, cycloheteroalkyl, or substituted cycloheteroalkyl as defined.

"Alkylthio" denotes the group —SR, —$S(O)_{n=1-2}$—R, where R is lower alkyl, substituted lower alkyl, aryl, substituted aryl, aralkyl or substituted aralkyl as defined herein.

"Acyl" denotes groups —C(O)R, where R is hydrogen, lower alkyl substituted lower alkyl, aryl, substituted aryl and the like as defined herein.

"Aryloxy" denotes groups —OAr, where Ar is an aryl, substituted aryl, heteroaryl, or substituted heteroaryl group as defined herein.

"Amino" denotes the group NRR', where R and R' may independently by hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, hetaryl, or substituted hetaryl as defined herein or acyl.

"Amido" denotes the group —C(O)NRR', where R and R' may independently by hydrogen, lower alkyl, substituted lower alkyl, aryl, substituted aryl, hetaryl, substituted hetaryl as defined herein.

"Carboxyl" denotes the group —C(O)OR, where R is hydrogen, lower alkyl substituted lower alkyl, aryl, substituted aryl, hetaryl, and substituted hetaryl as defined herein.

"Aryl"—alone or in combination means phenyl or naphthyl optionally carbocyclic fused with a cycloalkyl of preferably 5–7, more preferably. 5–6, ring members and/or optionally substituted with 1 to 3 groups or substituents of halo, hydroxy, alkoxy, alkylthio, alkylsuifinyl, alkylsulfonyl, acyloxy, aryloxy, heteroaryloxy, amino optionally mono- or di-substituted with alkyl, aryl or heteroaryl groups, amidino, urea optionally substituted with alkyl, aryl, heteroaryl or heterocyclyl groups, aminosulfonyl optionally N-mono- or N,N-di-substituted with alkyl, aryl or heteroaryl groups, alkylsulfonylamino, arylsulfonylamino, heteroarylsulfonylamino, alkylcarbonylamino, arylcarbonylamino, heteroarylcarbonylamino, or the like.

"Substituted aryl" refers to aryl optionally substituted with one or more functional groups, e.g., halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Heterocycle" refers to a saturated, unsaturated, or aromatic carbocyclic group having a single ring (e.g., morpholino, pyridyl or furyl) or multiple condensed rings (e.g., naphthpyridyl, quinoxalyl, quinolinyl, indolizinyl or benzo[b]thienyl) and having at least one hetero atom, such as N, O or S, within the ring, which can optionally be unsubstituted or substituted with, e.g., halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Heteroaryl"—lone or in combination means a monocyclic aromatic ring structure containing 5 or 6 ring atoms, or a bicyclic aromatic group having 8 to 10 atoms, containing one or more, preferably 1–4, more preferably 1–3, even more preferably 1–2, heteroatoms independently selected from the group O, S, and N, and optionally substituted with 1 to 3 groups or substituents of halo, hydroxy, alkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, acyloxy, aryloxy, heteroaryloxy, amino optionally mono- or di-substituted with alkyl, aryl or heteroaryl groups, amidino, urea optionally substituted with alkyl, aryl, heteroaryl or heterocyclyl groups aminosulfonyl optionally N-mono- or N,N-di-

substituted with alkyl, aryl or heteroaryl groups, alkylsulfonylamino, arylsulfonylamino, heteroarylsulfonylamino, alkylcarbonylamino, arylcarbonylamino, heteroarylcarbonylamino, or the like. Heteroaryl is also intended to include oxidized S or N, such as sulkily, sulfonyl and N-oxide of a tertiary ring nitrogen. A carbon or nitrogen atom is the point of attachment of the heteroaryl ring structure such that a stable aromatic ring is retained. Examples of heteroaryl groups are pyridnyl, pyridazinyl, pyrazinyl, quinazolinyl, purinyl, indolyl, quinolinyl, pyrpmdinyl, pyrrolyl, oxazolyl, thiazolyl, thienyl, isoxazolyl, oxathiadiazolyl, isothiazolyl, tetrazolyl, imidazolyl, triazinyl, furanyl, benzofuryl, indolyl and the like. A substituted heteroaryl contains a substituent attached at an available carbon or nitrogen to produce a stable compound.

"Heterocyclyl"—alone or in combination means a non-aromatic cycloalkyl group having from 5 to 10 atoms in which from 1 to 3 carbon atoms in the ring are replaced by heteroatoms of O, S or N, and are optionally benzo fused or fused heteroaryl of 5–6 ring members and/or are optionally substituted as in the case of cycloalkyl. Heterocycyl is also intended to include oxidized S or N, such as sulfinyl, sulfonyl and N-oxide of a tertiary ring nitrogen. The point of attachment is at a carbon or nitrogen atom. Examples of heterocyclyl groups are tetrahydrofulranyl, dihydropyridinyl, piperidinyl, pyrrolidinyl, piperazinyl, dihydrobenzofuryl, dihydroindolyl, and the like. A substituted hetercyclyl contains a substituent nitrogen attached at an available carbon or nitrogen to produce a stable compound.

"Substituted heteroaryl" refers to a heterocycle optionally mono or poly substituted with one or more functional groups, e.g., halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Aralkyl" refers to the group —R—Ar where Ar is an aryl group and R is lower alkyl or substituted lower alkyl group. Aryl groups can optionally be unsubstituted or substituted with, e.g., halogen, lower alkyl, alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Heteroalkyl" refers to the group —R-Het where Het is a heterocycle group and R is a lower alkyl group. Heteroalkyl groups can optionally be unsubstituted or substituted with e.g., halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Heteroarylalkyl" refers to the group —R-HetAr where HetAr is an heteroaryl group and R lower alkyl or substituted lower alkyl. Heteroarylalkyl groups can optionally be unsubstituted or substituted with, e.g., halogen, lower alkyl, substituted lower alkyl, alkoxy, alkylthio, acetylene, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Cycloalkyl" refers to a divalent cyclic or polycyclic alkyl group containing 3 to 15 carbon atoms.

"Substituted cycloalkyl" refers to a cycloalkyl group comprising one or more substituents with, e.g., halogen, lower alkyl, substituted lower alkyl, alkoxy, alkylthio, acetylene, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Cycloheteroalkyl" refers to a cycloalkyl group wherein one or more of the ring carbon atoms is replaced with a heteroatom (e.g., N, O, S or P).

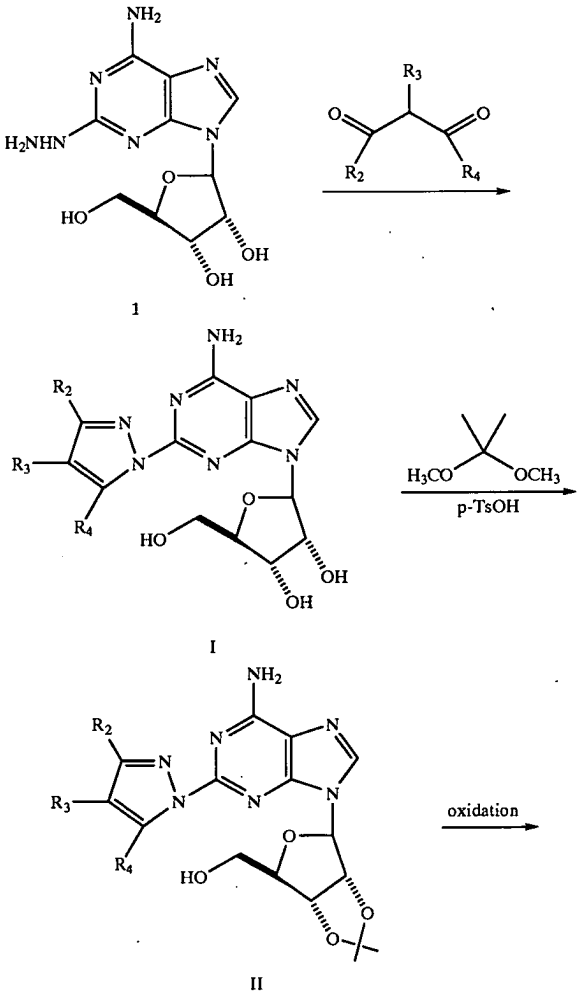
"Substituted cycloheteroalkyl" refers to a cycloheteroalkyl group as herein defined which contains one or more substituents, such as halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Alkyl cycloalkyl" denotes the group —R-cycloalkyl where cycloalkyl is a cycloalkyl group and R is a lower alkyl or substituted lower alkyl. Cycloalkyl groups can optionally be unsubstituted or substituted with e.g. halogen, lower alkyl, lower alkoxy, alkylthio, acetylene, amino, amido, carboxyl, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

"Alkyl cycloheteroalkyl" denotes the group —R-cycloheteroalkyl where R is a lower alkyl or substituted lower alkyl. Cycloheteroalkyl groups can optionally be unsubstituted or substituted with e.g. halogen, lower alkyl, lower alkoxy, alkylthio, amino, amido, carboxyl, acetylene, hydroxyl, aryl, aryloxy, heterocycle, substituted heterocycle, hetaryl, substituted hetaryl, nitro, cyano, thiol, sulfamido and the like.

The compounds of this invention can be prepared as outlined in Schemes 1–4. Compounds having the general formula IV can be prepared as shown in Scheme 1.

Scheme 1.



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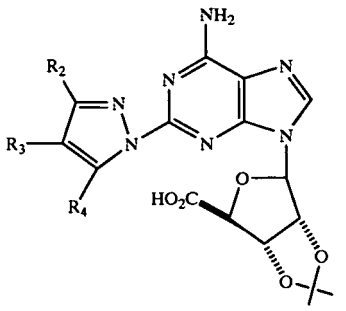
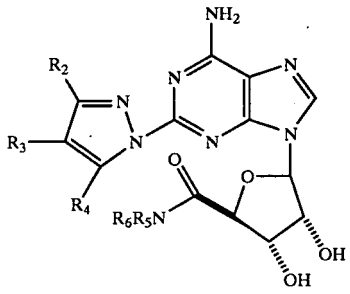


III

1) R_6R_5N, coupling
2) acid



IV

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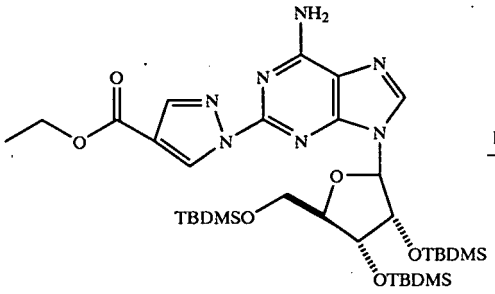
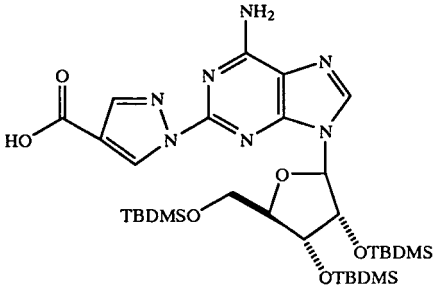


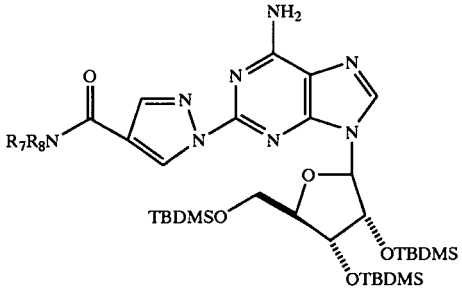
3

hydrolyze



4

couple

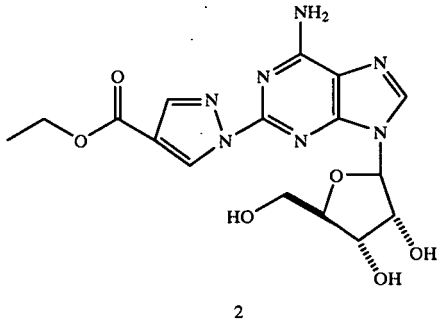


V

Compound I can be prepared by reacting compound I with appropriately substituted 1,3-dicarbonyl in a mixture of AcOH and MeOH at 80° C. (Holzer et al., J. Heterocycl. Chem. (1993) 30, 865). Compound II, which can be obtained by reacting compound I, with 2,2-dimethoxypropane in the presence of an acid, can be oxidized to the carboxylic acid III, based on structurally similar compounds using potassium permanganate or pyridinium chlorocbromate (M. Hudlicky, (1990) Oxidations in Organic Cherity ACS Monographs, American Chemical Society, Washington D.C.). Reaction of a primary or secondary amine having the formula HNR^6R^7, and compound MI using DCC (M. Fujino et al., Chem. Pharm. Bull. (1974), ^{22,1}i857), PyBOP (J. Martinez et al., J. Med. Chem. (1988) 28, 1874) or PyBrop (3. Caste et al. Tetrahedron, (1991), 32, 1967) couplings conditions can afford compound IV.

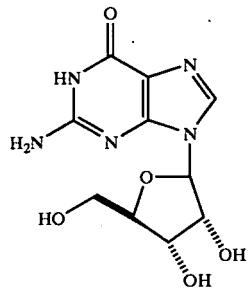
Compound V can be prepared as shown in Scheme 2. The Tri TBDMS derivative 4 can be obtained by treating compound 2 with TBDMSCl and imidazole in DMF followed by hydrolysis of the ethyl ester using NaOH. Reaction of a primary or secondary amine with the formula HNR^6R^7, and compound 4 using DCC (M. Fujino et al., Chem. Pharm. Bull. (1974), 22, 1857), PyBOP (J. Martinez et al., J. Med. Chem. (1988) 28, 1874) or PyBrop (J. Caste et al. Tetrahedron, (1991), 32, 1967) coupling conditions can afford compound V.

Scheme 2.



2

protect

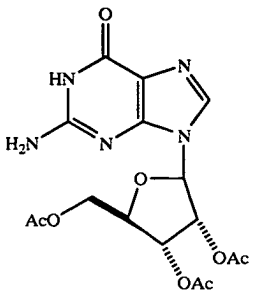


5

Scheme 3

Ac_2O

-continued



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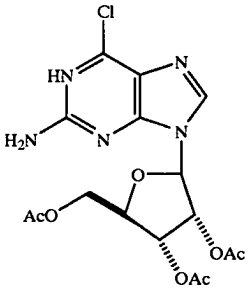

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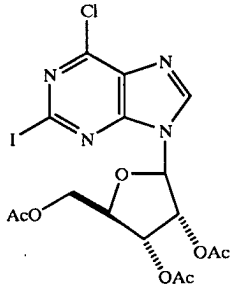

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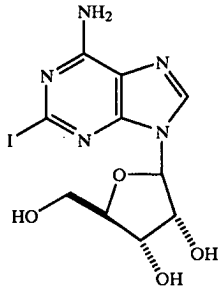

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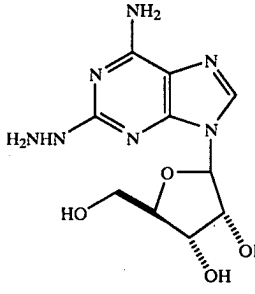
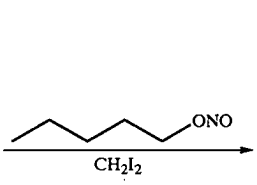
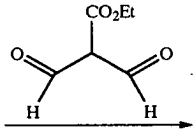


1





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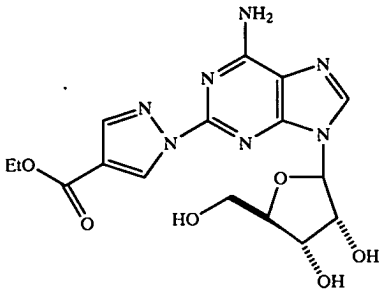

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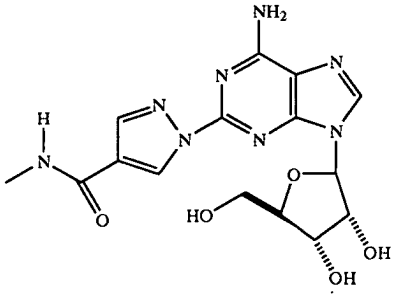
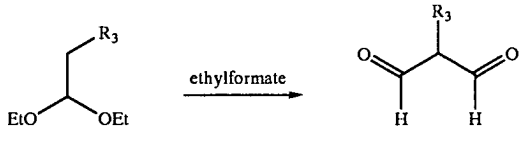


11

A specific synthesis of Compound 11 is illustrated in Scheme 3. Commercially available guanosine 5 was converted to the triacetate 6 as previously described (M. J. Robins and B. Uznanski, Can. J. Chem. (1981), 59, 2601–2607). Compound 7, prepared by following the literature procedure of Cerster et al. (J. F. Cerster, A. F. Lewis, and R. K. Robins, Org. Synthesis, 242–243), was converted to compound 9 in two steps as previously described (V. Nair et al., J. Org. Chem., (1988), 53, 3051–3057). Compound 1 was obtained by reacting hydrazine hydrate with compound 9 in ethanol at 80° C. Condensation of compound 1 with ethoxycarbonylmalondialdehyde in a mixture of AcOH and MEOH at 80° C. produced compound 10. Heating compound 10 in excess methylamine afforded compound 11. Scheme 4

Scheme 4



VI VII

The synthesis of 1,3-dialdehyde VII is described in Scheme 4. Reaction of 3,3-diethoxypitrite or 3,3-diethoxypropionitrile or 1,-diethoxy-2-nitroethane VI (R_3 =CO_2R, CN or NO_2) with ethyl or methyl formate in the presence of NaH can afford the dialdehyde VII (Y. Yamamoto et al., J. Org. Chem. (1989) 54, 4734).

Compounds of this invention are useful in conjunction with radioactive imaging agents to image coronary activity. The compounds of this invention are A_{2A} agonists that are believed to provide specific activation of adenosine A_{2A} receptors in the coronary vessels as opposed to adenosine Al receptors in the atrium and AV-node and/or A_2B 1A, receptors in peripheral vessels, thus avoiding undesirable side-

effects. Upon administration in a therapeutic amount, the compounds of this invention cause coronary blood vessels to vasodilate to induce coronary steal wherein healthy coronary vessels steal blood from unhealthy vessels resulting in lack of blood flow to heart tissues. Lower doses of the A_{2A} agonists may provide beneficial coronary vasodilatation (less severe) in the treatment of chronic CAD.

As A_{2A} agonists, the compounds of this invention are also useful in adjunctive therapy with angioplasty to induce dilation, inhibit platelet aggregation, and as a general anti-inflammatory agent. A_{2A} agonists, such as the compounds of this invention, can provide the therapeutic benefits described above by preventing neutrophil activation (Purinergic Approaches in Experimental Therapeutics K. A. Jacobson and M. F. Jarvis 1997 Wiley, New York). The compounds of this invention are also effective against a condition called no-reflow in which platelets and neutrophils aggregate and block a vessel. As A_{2A} 1A, agonists, the compounds of this invention are effective against no-reflow by preventing neutrophil and platelet activation (e.g., they are believed to prevent release of superoxide from neutrophils). As A_{2A} agonists, the compounds of this invention are also useful as cardioprotective agents through their anti-inflammatory action on neutrophils. Thus, in situations when the heart will go through an ischemic state such as a transplant, they will be useful.

This invention also includes pro-drugs of the above-identified A_{2A} agonists. A pro-drug is a drug which has been chemically modified and may be biological inactive at its site of action, but which will be degraded or modified by one or more enzymatic or in vivo processes to the bioactive form. The pro-drugs of this invention should have a different pharmacokinetic profile to the parent enabling improved absorption across the mucosal epithelium, better salt formulation and/or solubility and improved systemic stability. The above-identified compounds may be preferably modified at one or more of the hydroxyl groups. The modifications may be (1) ester or carbonate derivatives which may be cleaved by esterases or lipases, for example; (2) peptides which may be recognized by specific or non specific proteinase; or (3) derivatives that accumulate at a site of action through membrane selection or a pro-drug form or modified pro-drug form, or any combination of (1) to (3) above.

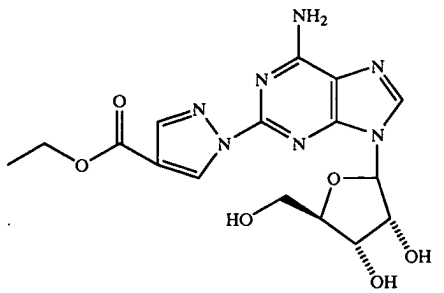
The compounds may be administered orally, intravenously, through the epidermis or by any other means known in the art for administering a therapeutic agents. The method of treatment comprises the administration of an effective quantity of the chosen compound, preferably dispersed in a pharmaceutical carrier. Dosage units of the active ingredient are generally selected from the range of 0.01 to 100 mg/kg, but will be readily determined by one skilled in the art depending upon the route of administration, age and condition of the patient. This dose is typically administered in a solution about 5 minutes to about an hour or more prior to coronary imaging. No unacceptable toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

If the final compound of this invention contains a basic group, an acid addition salt may be prepared Acid addition salts of the compounds are prepared in a standard manner in a suitable solvent from the parent compound and an excess of acid, such as hydrochloric, hydrobromic, sulfuinc, phosphoric, acetic, maleic, succinic, or methanesulfonic. The hydrochloric salt form is especially useful. If the final compound contains an acidic group, cationic salts may be prepared. Typically the parent compound is treated with an excess of an alkaline reagent, such as hydroxide, carbonate or alkoxide, containing the appropriate cation. Cations such as Na^+, K^+, Ca^{+2} and NH_4^+ are examples of cations present in pharmaceutically acceptable salts. Certain of the compounds form inner salts or zwitterions which may also be acceptable.

Pharmaceutical compositions including the compounds of this invention, and/or derivatives thereof, may be formulated as solutions or lyophilized powders for parenteral administration. Powders may be reconstituted by addition of a suitable diluent or other pharmaceutically acceptable carrier prior to use. If used in liquid form the compositions of this invention are preferably incorporated into a buffered, isotonic, aqueous solution. Examples of suitable diluents are normal isotonic saline solution, standard 5% dextrose in water and buffered sodium or ammonium acetate solution. Such liquid formulations are suitable for parenteral administration, but may also be used for oral administration. It may be desirable to add excipients such as polyvinylpyrrolidinone, gelatin, hydroxycellulose, acacia, polyethylene glycol, mannitol, sodium chloride, sodium citrate or any other excipient known to one of skill in the art to pharmaceutical compositions including compounds of this invention. Alternatively, the pharmaceutical compounds may be encapsulated, tableted or prepared in an emulsion or syrup for oral administration. Pharmaceutically acceptable solid or liquid carriers may be added to enhance or stabilize the composition, or to facilitate preparation of the composition. Liquid carriers include syrup, peanut oil, olive oil, glycerin, saline, alcohols and water. Solid carriers include starch, lactose, calcium sulfate, dihydrate, teffa alba, magnesium stearate or stearic acid, talc, pectin, acacia, agar or gelatin. The carrier may also include a sustained release material such as glycerol monostearate or glycerol distearate, alone or with a wax. The amount of solid carrier varies but, preferably, will be between about 20 mg to about 1 gram per dosage unit. The pharmaceutical dosages are made using conventional techniques such as milling, mixing, granulation, and compressing, when necessary, for tablet forms; or milling, mixing and filling for hard gelatin capsule forms. When a liquid carrier is used, the preparation will be in the form of a syrup, elixir, emulsion or an aqueous or non-aqueous suspension. Such a liquid formulation may be administered directly or filled into a soft gelatin capsule. It is preferred that the compositions of this invention are administered as a solution either orally or intravenously by continues infusion or bolus.

The Examples which follow serve to illustrate this invention. The Examples are intended to in noway limit the scope of this invention, but are provided to show how to make and use the compounds of this invention. In the Examples, all temperatures are in degrees Centigrade.

EXAMPLE 1

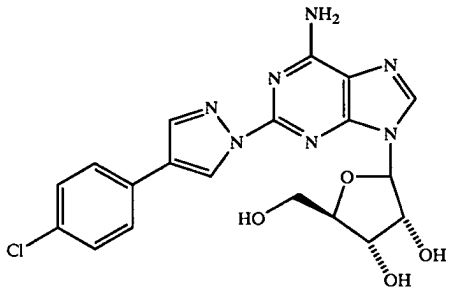


Ethyl1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylate (12)

To a suspension of 2-hydrazinoadenosine (0.025 g, 0.08 mmol) in a 1:1 mixture of MeOH/AcOH was added

(ethoxycarbonyl)malondialdehyde ((0.019 g, 0.12 mmol) and the mixture was heated heated at 80° C. for 3 h. The precipitate formed was collected by filtration and washed with EtOH and ether to afford 12. ^{1}HNMR (DMSO-d6) δ 1.25 (t, 3H), 3.5 (m, 1 H), 3.6 (m, 1H), 3.8 (d, 1H), 4.15 (d, 1H), 4.55 (m, 1H), 5.0 (t, 1H), 5.2 (d, 1H), 5.5 (d, 1H), 5.5 (d, 1H), 5.9 (d, 1H), 7.15–7.3 (m, 5H), 7.8 (br s, 2H), 8.1 (s, 1H), 8.4 (s, 1H,) 8,9(s, 1H).

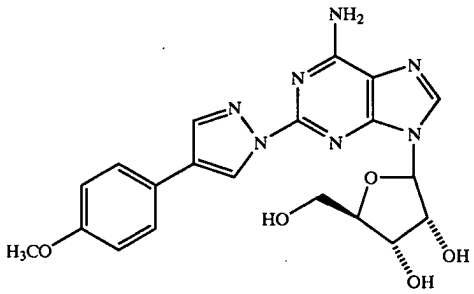
EXAMPLE 2



(4S,2R,3R,5R)-2-{-Amino-2-[4-(4-chlorophenyl) pyrazolyl]purin-9-yl}-5-(hydroxymethyl)oxolane-3, 4-diol (13)

To a suspension of 2-hydrazinoadenosine (0.025 g, 0.08 mmol) in a 1:1 mixture of MeOH/AcOH was added 2-(4-chloro)phenylmalondialdehyde (0.022g, 0.12 mmol) and the mixture was heated at 80° C. for 3 h. The precipitate formed was collected by filtration and washed with EtOH and Ether to afford 13. ^{1}HNMR (DMSO-d6) δ 3.5 (m, 1H), 3.6 (m, 1H), 3.8 (d, 1H), 4.15 (d, 1H), 4.2 (q, 2H), 4.55 (m, 1H), 5.9 (d, 1H), 7.45 (d, 2H), 7.75 (d, 2H)8.25 (s, 1H), 8.35 (s, 1H), 8.9 (s, 1H).

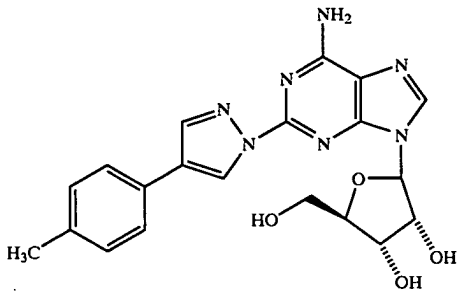
EXAMPLE 3



(4S,2R,3R,5R)-2-{6-Amino-2-[4-(4-metboxyphenyl) pyrazolyl]purin-9-yl}-5-(hydroxymethyl)oxolane-3, 4-diol (14)

To a suspension of 2-hydrazinoadenosine (0.025 g, 0.08 mmol) in a 1:1 mixture of MeOH/AcOH was added 2-(4-methoxy)phenylmalondialdehyde (0.022g, 0.12 mmol) and the mixture was heated at 80° C. for 3 h. The precipitate formed was collected by filtration and washed with EtOH and Ether to afford 14. ^{1}HNMR (DMSO-d6) δ 3.55 (m, 1H), 3.65 (m, 1 H), 3.75 (s, 3H), 3.9 (d, 1H), 4.15 (d, 1H), 4.6 (m, 1H), 5.9 (d, 1H), 6.75 (d, 2H), 7.6 (d, 2H),8.15 (s, 1H), 8.35 (s, 1H), 8.8 (s, 1H).

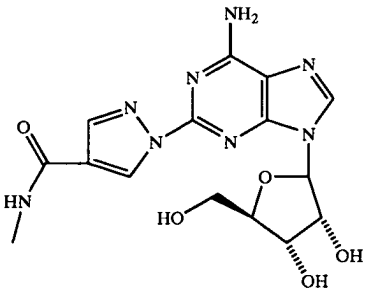
EXAMPLE 4



(4S,2R,3R,5R)-2-{6-Amino2-[4-(4-methylphenyl) pyrazolyl]purin-9-yl}-5-(hydroxymethyl)oxolane-3, 4-diol (15)

To a suspension of 2-hydrazinoadenosine (0.025 g, 0.08 mmol) in a 1:1 mixture of MeOH/AcOH was added 2-(4-methyl)phenylmalondialdehyde (0.019 g, 0.12 mmol) and the mixture was heated at 80° C. for 3 h. The precipitate formed was collected by filtration and washed with EtOH and Ether to afford 15. ^{1}HNMR (DMSO-d6) δ 3.55 (m, 1H\), 3.65 (m, 1H), 3.75 (s, 3H), 3.9 (d, 1H), 4.15 (d, 1H), 4.6 (m, 1H), 5.9 (d, 1H), 6.75 (d, 2H), 8.15 (s, 1H), 8.35 (s, 1H), 8.8 (s, 1H).

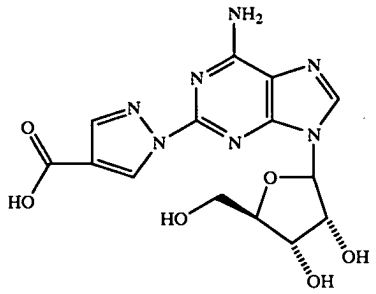
EXAMPLE 5



(1-{9-[(4S,2R,3R,-5R)-3,4-dihydroxy-5-(bydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide (16)

Compound 12 (0.05 mg, 0.12 mmol) was added to 4 mL methylamine (40% sol. In water). The mixture heated at 65° C. in for 24 h. After concentration in vacuo, the residue was purified using prep. TLC (10% MeOH:DCM). ^{1}HNMR (CD_3OD) δ 2.90 (s, 3H), 3.78 (m, 1H), 3.91 (m, 1H), 4.13 (d, 1H), 4.34 (d, 1H), 4.64 (m, 1H), 6.06 (d, 1H), 8.38 (s, 1H), 9.05 (s, 1H).

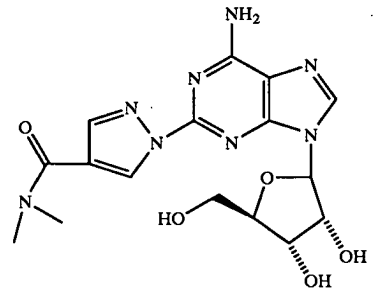
EXAMPLE 6



1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylic Acid (17)

Compound 12 (0.05 mg, 0.12 mmol) was dissolved one equivalent of 1N NaOH. The solution was allowed to stir at Rt for 2 h, then acidified to pH 4. The resulting precipitate was filtered and washed with water and ether. ^{1}HNMR (CD_3OD) Δ3.75 (m, 1H), 3.90 (m, 1H), 4.13 (d, 1H), 4.43 (d, 1H), 4.64 (m, 1H), 6.05 (d, 1H), 8.10 (s, 1H), 8.35 (s, 1H), 9.05 (s, 1H).

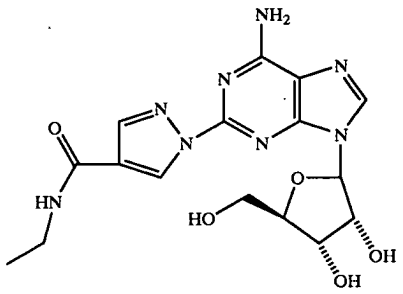
EXAMPLE 7



(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N,N-dimethylcarboxamide (18)

Compound 18 was prepared in a manner similar to that of compound 16 using dimethylamine instead of methylamine, MS 405.12 (M+1).

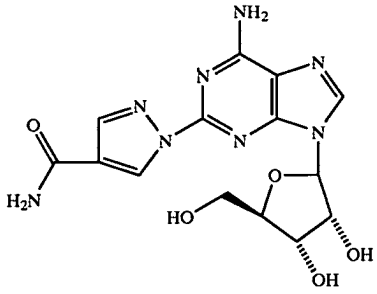
EXAMPLE 8



(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydrozymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-ethylcarboxamide (19)

Compound 19 was prepared in a manner similar to that of compound 16 using ethylamine instead of methylamine, MS 405.35 (M+1).

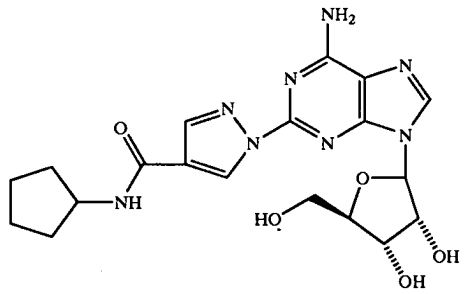
EXAMPLE 9



1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl }pyrazole-4-carboxamide (20)

Compound 20 was prepared in a manner similar to that of compound 16 using ammonia instead of methylamine, MS 377.25 (M+1).

EXAMPLE 10

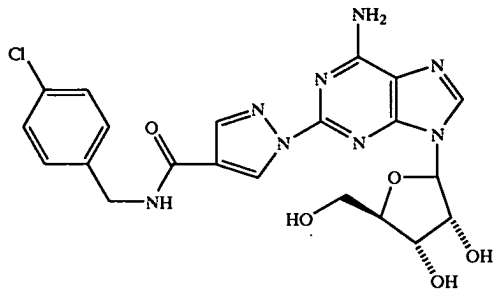


(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-(cyclopentyl)carboxamide (21)

Compound 12 (0.5 g, 1.2 mmol) was dissolved in dry DMF, TBDMSCl (1.5 g, 10 mmol) and imidazole (0.68 g, 10 mmol) were added and the mixture was heated at 80° C. for 24 h. The solvent was evaporated and the residue was purified by flash column to obtain the trisilyl protected form of compound 12. The trisilyl derivative (0.8 g) was then suspended in 1 mL of water and treated with 2 m L1N KOH/MeOH. The mixture was stirred at RT for 72. The solvent was removed under reduced pressure and the residue was suspended in 5 mL of water and acidified to pH 5.5 with 1N HCl. The resulting precipitate was filtered and washed with water and ethyl ether to afford the trisilyl form of the acid 20.

The trisilyl derivative acid 20 (0.14 g, 0.2 mmol) was then dissolved in 5 mL dichloromethane. To the solution was added HBTU (0.19 g, 0.4 mmol), HOBt (0.076 g, 4 mmol), N-methylmorpholine (0.04 g, 0.4 mmol) and cat. DMAP. The mixture was allowed to stir at RT for 24 h. The mixture was then washed with 10% citric acid, saturated $NaHCO_3$, brine and dried over $MgSO_4$. The solvent was removed and the residue was treated with 5 mL 0.5N NH_4F/MeOH. The solution was heated at reflux for 24 h. The solvent was evaporated and the residue was purified by preparative TLC to afford compound 21, MS 445.26 (M+1).

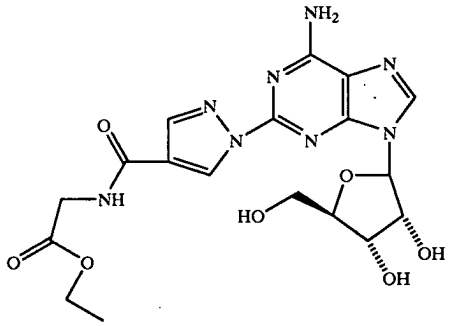
EXAMPLE 11



(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2yl}pyrazol-4yl)-N-[(4-chlorophenyl)methyl] carboxamide (22)

Compound 22 was prepared in a manner similar to that of compound 21 using 4 chlorobenzylamine instead of cyclopentylamine, MS 501.19 (M+1).

EXAMPLE 13



Ethyl 2-[(1-{9-(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymetbyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)carbonylaminolacetate (23)

Compound 23 was prepared in a manner similar to that of compound 21 using glycine methyl ester instead of cyclopentylamine, MS 445.26 (M+1).

EXAMPLE 14

Compounds of this invention were assayed to determine their affinity for the A_{2A} receptor in a pig striatum membrane prep. Briefly, 0.2 mg of pig striatal membranes were teated with adenosine deaminase (2 U/mL) and 50 mM Tris buffer (pH=7.4) followed by mixing. To the pig membranes was added 2 μL of serially diluted DMSO stock solution of the compounds of this invention at concentrations ranging from 10 nM to 100 microM or the control received 2 microL of DMSO alone, then the antagonist ZM 241385 in Tris buffer (50 mM, pH of 7.4) was added to achieve a final concentration of 2nM . After incubation at 23> C. for 2 h, then the solutions were filtered using a membrane harvester using multiple washing of the membranes (3×). The filter disks were counted in scintillation cocktail to determine the amount of displacement of tritiated ZM displaced by the compounds of this invention. Greater than a 5 point curve was used to generate Ki.'s and the number of experiments is indicated in the column marked in Table 1 below.

TABLE 1

Compound Number	A_{2a} Ki, nM	n
12	+++	2
13	++	3
14	++	1
15	++	3
16	++	2
17	-	1
18	+++	3
19	+++	3
20	+++	3
21	+++	3
22	+++	3

+++ = 10–1,000 nM
++ = 1,000–10,000 nM
+ = greater than 10,000 nM
- = greater than 100,000 nM

EXAMPLE 15

The objective of this experiment was to determine the affinities and receptor binding selectivity of a compound of this invention for A_1, A_{2A}, A_{2B} and A_3 adenosine receptors. Molecular cloning has identified and confirmed the existence of four subtypes of adenosine receptors (AdoRs), designated as A_1, A_{2A}, $A_{2B\ and\ A3}$AdoRs (Linden, 1994). These AdoR subtypes have distinct anatomical distributions, pharmacological properties and physiological functions (Shryock and Belardinelli, 1997). A_1 and A_3AdoRs couple to inhibitory G proteins ($G_i/_o$) and decrease the activity of adenylyl cyclase, whereas A_{2A} and A_{2B}AdoRs increase intracellular cAMP content via coupling to stimulatory G proteins (Gs).

Ligands with high potency and tissue/organ selectivity for distinct adenosine receptor subtypes have therapeutic and diagnostic potentials for a variety of diseases (such as arrhythmia, ischemic heart diseases, asthma and Parlinson's disease) and are the focus of considerable research efforts by both academia and industry. Here we report the pharmacological and functional characterization of a series of novel adenosine analogues of this invention using mammalian cell lines expressing either endogenous AdoRs or recombinant human AdoRs.

Materials

Adenosine deaminase was purchased from Boehringer Mannheim Biochemicals Indianapolis, Id., U.S.A). [^{3}H] ZM241385 (Lot No. 1) was purchased from Tocris Cookson Ltd (Langford, Bristol, UK). [^{3}H]CPX (Lot No. 3329207) was from New England Nuclear (Boston, Mass., USA). CGS21680 (Lot No. SW-3R-84 and 89H4607), NECA (Lot No. OXV-295E), R-PIA (Lot No. WY-V-23), Rolipram and HEK-hA_2AAR membranes were obtained from Sigma-RBI (Natick, Mass.). WRC-0470 was prepared as described in the literature (K. Nuiya et al., J. Med. Chem. 35; 4557–4561 (1992); Compound 16 of this invention was synthesized as described above and prepared as a stock solution (10 mmol/L) in DMSO.

Cell culture and membrane preparation—PC12 cells were obtained from the American Type Culture Collection and grown in DMEM with 5% fetal bovine serum, 10% horse serum, 0.5 mmol/L L-glutamine, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 2.5 μg/mL amphotericin. HEK-293 cells stably expressing recombinant human A_{2B}AdoRs (HEK-hA_{2B}AdoR) were grown in DMEM supplemented with 10% fetal bovine serum and 0.5 mg/mL G-418. CHOK1 cells stably expressing the recombinant human A_1 AdoR (CHO-hA_1AdoR) and A_3AdoR (CHO-hA_3AdoR)

were grown as monolayers on 150-mm plastic culture dishes in Ham's F-12 media supplemented with 10% fetal bovine serum in the presence of 0.5 mg/mL G-418. Cells were cultured in an atmosphere of 5% CO_2/95% air maintained at 37° C.

To make membranes, cells were detached from the culture plates into ice-cold 50 mmol/L Tris-HCl buffer (pH7.4). The cell suspensions were homogenized with Polytron at setting 4 for 30 seconds, and spun at 48,000g for 15 minutes. The pellets were washed three times by re-suspension in ice-cold Tris-HCl buffer and centrifugation. The final pellet was re-suspended in a small volume of Tris-HCl, aliquoted and frozen at −80° C. until used for receptor binding assays. The protein concentration of membrane suspensions was determined using the Bradford method (Bio-Rad) with bovine serum as standards.

Competition Binding Assays—Competition assays were performed to determine the affinities (K_i) of the following unlabeled compounds (competing agents): Compounds WRC-0470; Compound 16 of this invention, NECA, CGS 21680 and R-PIA for A_1 AdoRs (R[^{3}H]DPCPX binding sites on CHO-hA_1AdoR cell membranes), A_{2A} AdoRs(R[^{3}H] ZM241385 binding sites on PC12 and HEK-hA_{2A}AR cell membranes),

A_{2B}AdoR (R[^{3}H]DPCPX binding sites on HEK-h$_{A2}$AdoR cell membranes) and A_3AdoR (R[^{125}I]ABMECA binding sites on CHO-hA_3AdoR cell membrane).

Membrane suspensions were incubated for 2 hours at room temperature in 50 mmol/L Tris-HCl buffer (pH 7.4) containing ADA (1 U/mL), Gpp(NH)p (100 μM), radioligand {either [^{3}H]ZM241385 (−1.5 to 5 mmol/L), [^{3}H] DPCPX (~2.5 to 3.0 mmol/L for A_1 and 30 nM for A_{2B}) or [^{125}]ABMECA (1 nM)} and progressively higher concentrations of the competing agents. At the end of incubation, bound and free radioligands were separated by filtration through Whatman GF/C glass fiber filters using a Brandel tissue harvester (Gaithersburg, Md.). Triplicate determinations were performed for each concentration of the competing agent.

Study Design (Protocols)

The affinity (KY) of various CVT compounds for the A_1 and A_{2A} adenosine receptor were determined by their potency to compete for [^{3}H]CPX (A_1) or [3H]ZM241385 (A_{2A}) binding sites on membranes derived from CHO-hA_1AdoR, PC12 or HEK-HA_{2A}AdoR cells. R-PIA and CGS21680, agonists that are selective for A_1 and A_{2A} respectively, and NECA, a non-selective AdoR agonist were used as controls. To facilitate comparison and avoid the complication of multiple affinity states due to receptor coupling to G-proteins, the competition binding studies were carried out in the presence of Gpp (NH) p (100 μM) to uncouple receptors from G-proteins. The affinity of selected compounds for A_{2A} and A_3 receptors were assessed by their potencies to compete for [^{3}H] CPX (A_{2A}) and [^{125}I] ABMECA (A3) binding sites on membranes derived from HEK-hA_{2A}AdoR and CHO-hA_3AdoR cells, respectively.

Results

The affinity (K_i) of WRC-0470; and Compound 16 for human A_1, rat and human A_{2A}AdoRs, as determined by competition binding studies are summarized in Table 2, below. All compounds show moderate selectivity for human A_{2A} versus A_1 receptor. Furthermore, Compound 16, at a concentration of 10 μM, decreased the specific binding of [^{3}H] CPX (HEK-hA_{2B}AdoR) or [^{125}I] IBMECA (CHO-hA_3AdoR) by 20% and 22%, respectively.

TABLE 2

Binding Affinities of Adenosine Receptor Agonists for A_{2A}AdoRs and A_1AdoRs
K_i/nmol/L (pK_i ± SEM)

	HEK-hA_{2A}AR Cells		CHO-hA_1AR	
	Binding Affinity	n	Binding Affinity	n
WRC-0470	272 (6.55 ± 0.04) [0.83 ± 0.07]	6	7278 (5.16'± 0.09) [1.13 ± 0.21]	3
Compound 16	1269 (5.90 ± 0.03) [0.73 ± 0.04]	7	>16460 (4.59 ± 0.35) [0.92 ± 0.04]	3
CGS21680	609 (6.22 ± 0.06) {0.65 ± 0.07)	3	>3540 (5.47 ± 0.20)	3
NECA	360 (6.45 ± 0.06) [0.83 ± 0.08]	3	328 (6.49 ± 0.06) [0.88 ± 0.03]	3
R-PIA	1656 (5.78 ± 0.02) [1.05 ± 0.02)	3	477 (6.35 ± 0.11) [1.03 ± 0.08)	3

The results of this Experiment show that Compound 16 is a low affinity A_{2A} agonist.

EXAMPLE 16

The objective of this Example was to characterize pharmacologically the effects of Compound 16 of this invention on coronary artery conductance. Specifically, the experiments were designed to determine 1) the potency Compound 16 and compared its potency to that of adenosine and other selected A_{2A} doR agonists, and 2) which adenosine receptor, the A_1 or A_{2A} AdoR subtype mediates the coronary vasodilation caused by Compound 16 of this invention.

In the heart, the A_{2A} adenosine receptor mediates the coronary vasodilation caused by adenosine, whereas the A_i receptor mediates the cardiac depressant actions of adenosine, such as the negative chronotropic and dromotropic (AV block) effects.

Several potent and selective ligands, both agonists and antagonists, for the A_1 and A_{2A} AdoRs have been synthesized. In the heart agonists of A_1 AdoRs have been proposed to be useful as antiarrhythmic agents, whereas agonists of A_{2A} AdoRs are being developed for selective coronary vasodilation

A series of adenosine derivatives targeted for selective activation of A_{2A}, adenosine receptor (A_{2A}doR) were synthesized for the purposes of developing coronary vasodilators. More specifically, in this study we report on the effect of a series of novel A_{2A} AdoR agonists on coronary artery conductance (vasodilation) in rat and guinea pig isolated perfused hearts.

Materials

Rats (Sprague Dawley) and Guinea pigs (Hartley) were purchased from Simonsen and Charles Rivers, respectively. WRC-0470 was prepared as described in the literature (K. Niiya et al., J. Med. Chem. 35; 4557–4561 (1992). Compound 16 of this invention was prepared as described above. CGS 21680 and adenosine were purchased from Sigma Krebs-Henseleit solution was prepared according to Standard Methods, and 0.9% saline was purchased from McGraw, Inc.

Methods

Adult Sprague Dawley rats and Hartley guinea-pigs of either sex weighing from 230 to 260 grams and 300 to 350 grams, respectively were used in this study. Animals were

anesthetized by peritoneal injection of a cocktail containing ketamine and xylazine (ketamine 100 mg, xylazine 20 mg/ml). The chest was opened and the heart quickly removed. The heart was briefly rinse in ice-cold Krebs-Henseleit solution (see below), and the aorta cannulated. The heart was then perfused at a flow rate of 10 ml/min with modified Krebs-Henseleit (K-H) solution containing NaCl 117.9, KCl 4.5, $CaCl_2$ 2.5, $MgSO_4$ 1.18, $KH_2PO_4$1–18, pyruvate 2.0 mmo/L. The K-H solution (pH 7.4) was gassed continuously with 95% 0.2 and 5% CO_2 and warmed to ±0.50° C. The heart was electrically paced at a fixed cycle length of 340 ms (250 beats/min) using a bipolar electrode place on the left atrium. The electrical stimuli were generated by a Grass stimulator (Model S48, W. Warwick, R.I.) and delivered through a Stimuli Isolation Unit (Model SIUS, Astro-Med, Inc., N.Y.) as square-wave pulses of 3-msec in duration and amplitude of at least twice the threshold intensity.

Coronary perfusion pressure (CPP) was measured using a pressure transducer, connected to the aortic cannula via a T-connector positioned approximately 3 cm above the heart Coronary perfusion pressure was monitored throughout the experiment and recorded either on a chart recorder (Gould Recorder ^{2200}S) or a computerized recording system (PowerLab/4S, ADinstruments Pty Ltd, Australia). Only hearts with CPP ranging from 60 to 85 mmHg (in the absence of drugs) were used in the study. Coronary conductance (in ml/min/mmHg) was calculated as the ratio between coronary perfusion rate (10 ml/min) and coronary perfusion pressure.

In experiments in which A_1 adenosine receptor-mediated negative dromotropic effect was measured, atrial and ventricular surface electrograms were recorded during constant atrial pacing. The effect of various adenosine receptor agonists on atrioventricular conduction time was determined as described previously by Jenkins and Belardinelli *Circ. Res.* 63:97–116 (1988).

Stock solutions of Compound 16 of this invention (5mM) and CGS 21680 (5mM) were prepared in dimethyl sulfoxide (DMSO); purchased from Aldrich, PS 04253MS. A stock solution of adenosine (1 mg/ml) was prepared in saline. One concentration was made from the stock solution by dilution into saline to yield solution of either 2×10^{4} or 2×10^{-5} M. These solutions were injected into the perfusion line of the apparatus as boluses of 20 μL. In some experiments the solutions were placed into a 30 ml glass syringe and the drugs were infused at rates necessary to achieve the desired perfusate concentrations (e.g, 10, 100 nM, etc).

Coronary Vasodilation of A_2 Adenosine Receptor Agonists

Concentration-response relationships for the effect of Compound 16 of this invention (0.1 to 400 nmM) and CGS21680 (0.1 to 10 nM) to increase coronary conductance were obtained. After control measurements of coronary perfusion pressure were recorded, progressive higher concentrations of the adenosine receptor agonists were administered until maximal coronary vasodilation was observed. The steady-state responses to each concentration of adenosine receptor agonists were recorded. In each heart of this series (4 to 6 hearts for each agonist) only one agonist and one concentration-response relationship was obtained

Coronary Vasodilatory Effect of Compound 16 in the Absence and Presence of Adenosine Receptor Antagonists.

To determine which adenosine receptor subtype (A_1 or A_{2A}) mediates the coronary vasodilation caused by Compound 12, the A_1 and A_{2A}, adenosine receptor antagonists CPX and ZM241385, respectively, were used. Hearts (n=6) were exposed to the compound being tested (10 nM), and after the effect of this agonist reached steady-state, first CPX (60 nM), and then ZM241385 were added to the perfusate and the changes in CPP were recorded.

In isolated perfused hearts (n=36 rats and 18 guinea pigs) paced at constant atrial cycle length of 340 msec, adenosine, CGS21680, WRC0470, and Compound 16 caused a concentration-dependent increase in coronary conductance. CGS21680 and WRC0470 were the most potent agonists tested. Compound 16 was approximately 10-fold more potent than adenosine to increase coronary conductance. It is worth noting that all agonists were several fold more potent coronary vasodilators in rat than guinea pig hearts (Table 3).

TABLE 3

Potency of Adenosine and A_{2A} Adenosine Receptor Agonists to Increase Coronary Conductance in Rat and Guinea Pig Isolated Perfused Hearts

		Potency (EC_{50})	
Agonist	n	Rat	Guinea Pig
Compound 16	4	6.4 ± 1.2	18.6 ± 6.0
Adenosine	4	59.2 ± 6.4	86.0 ± 0.5
CGS21680	4	0.5 ± 0.1	1.7 ± 0.4
WRC0470	3	0.6 ± 0.2	2.4 ± 1.1

To determine the AdoR subtype (A_1 versus A_{2A}) that is responsible for the coronary vasodilation observed in the presence of Compound 16, the effect of this agonist (10 nM) on coronary conductance was studied in the absence and presence of CPX, a selective A_{2A} AdoR antagonist (Belardinelli et al, 1998) and ZM241385, a selective $_{A2}$ AdoR antagonist (Poucher et al, 1 995) at the concentration of 60 nM. As shown in FIG. 1, Compound 16 significantly increased coronary conductance to 0.22+0.01 ml/mm Hg^{-1} min^{-} from a baseline value of 0.16+0.02 mlmmHg^{-1} min^{-1}. This increase in coronary conductance caused by Compound 16 was not affected by CPX but was completely reversed by ZM241385 (0.17±0.02 mlmm Hg-1 min^{-1}).

EXAMPLE 17

The objective of this Example was to determine the functional selectivity of Compound 16 to cause coronary vasodilation. Specifically, the potency of Compound 16 to cause coronary vasodilation (A_{2A} AdoR response) and prolongation of A-V nodal conduction time (A_1 AdoR response) were determined in rat and guinea pig hearts.

Materals

Sprague Dawley rats were purchased from Sirnonsen. Hartley guinea pigs were purchased from Charles River. Compound 16 was prepared as described above. CVT-510-2-{6[((3R)oxolan-3-yl)amino]purin-9-yl}(4S,3R,5R)-(hydroxymethyl)oxolane-3,4-diol—was prepared in accordance with the synthesis method disclosed in U.S. Pat. No. 5,789,416, the specification of which is incorporated herein by reference. Ketamine was purchased from Fort Dodge Animal Health (Lot No. 440444) and xylazine from Bayer (Lot No. 26051 A). Krebs-Henseleit solution was prepared according to the standard methods, and 0.9% sodium chloride was purchased from McGraw, Inc. (Lot No. J8B246).

Isolated Perfused Heart Preparation:

Rats and guinea pigs, of either sex weighing from 230 to 260 grams and 300 to 350 grams, respectively, were used in this study. Animals were anesthetized by peritoneal injection of a cocktail containing ketamine and xylazine (ketamine 100 mg, xylazine 20 mg/ml). The chest was opened and the

heart quickly removed. The heart was briefly rinse in ice-cold Krebs-Henseleit solution (see below), and the aorta cannulated. The heart was then perfused at a flow rate of 10 ml/min with modified Krebs-Henseleit (K-H) solution containing NaCl 117.9, KCl 4.5, $CaC_2$2.5, $MgSO_4$1.18, $KH_2PO_4$1.18, pyruvate 2.0 mmo/L. The K-H solution (pH 7.4) was gassed continuously with 95% ^{2} and 5% CO_2 and warmed to 35±0.50° C. The heart was electrically paced at a fixed cycle length of 340 ms (250 beats/min) using a bipolar electrode place on the left atrium. The electrical stimuli were generated by a Grass stimulator (Model S48, W. Warwick, R.I.) and delivered through a Stimuli Isolation Unit (Model SIU5, Astro-Med, Inc., N.Y.) as square-wave pulses of 3-msec in duration and amplitude of at least twice the threshold intensity.

Coronary perfusion pressure (CPP) was measured using a pressure transducer, connected to the aortic cannula via a T-connector positioned approximately 3 cm above the heart. Coronary perfusion pressure was monitored throughout the experiment and recorded either on a chart recorder (Gould Recorder 2200S) or a computerized recording system (PowerLab/4S, ADinstunents Pty Ltd, Australia). Only hearts with CPP ranging from 60 to 85 mmHg (in the absence of drugs) were used in the study. Coronary conductance (in ml/min/mmHg) was calculated as the ratio between coronary perfusion rate (10 ml/min) and coronary perfusion pressure.

A_1 adenosine receptor-mediated depression of A-V nodal conduction time (negative dromotropic effect) was measured. Atrial and ventricular surface electrograms in rats and His bundle electrogram in guinea pigs, were recorded during constant atrial pacing. The effects of Compound 16 on atrioventricular conduction time and stimulus-to-His-bundle (S-H interval) were determined as described previously by Jenkins and Belardinelli (1988).

The effects of Compound 16 on coronary conductance (A_{2A} effect) and atrioventricular conduction time or stimulus-to-His-bundle. (S-H) interval (A_1 ueffect) was then determined. Hearts were instrumented for continuous recording of coronary perfusion pressure (A_2A response) and atrioventricular (A-V) conduction time or S-H interval (A_1 response). In each experiment, concentration-response relationship of Compound 16 (n=6 rats, 4 guinea pigs) to increase coronary conductance and to prolong A-V conduction time or S-H interval was determined. After control measurements of CPP and A-V conduction time or S-H interval were made, progressive higher concentrations of Compound 16 was administered until maximal coronary vasodilation and A-V nodal conduction time or S-H interval prolongation were achieved. In separate rat hearts (n=4), the effect of various concentrations (100–400 nM) of CVT510, an A_1 adenosine agonist (Snowdy et al, 1999), on A-V nodal conduction time was determined and compared to that of Compound 16 (0.1–30 μM).

The concentration-response curves for Compound 16 to increase coronary artery conductance and to prolong A-V nodal conduction time or S-H internal are shown in FIGS. **2** and **3**. In both rat and guinea pig, Compound 16 increased coronary conductance in a concentration dependent manner. The potencies (EC_{50} values) for Compound 16 to increase coronary conductance in rat hearts was 6.4±0.6 nM and in guinea pig hearts was 18.6±6 OnM. In contrast, the effect of this agonist on S-H interval was somewhat variable between rat and guinea pig hearts. In rat hearts Compound 16 did not prolong A-V nodal conduction time (FIGS. **2** and **3**) whereas the A_1 AdoR agonist CVT510 significantly prolonged the A-V nodal conduction time (FIG. **4**). Unlike in rat, in guinea pig hearts Compound 16 caused a concentration-dependent prolongation of S-H interval (A1 response) with an EC_{50} value (potency) of 4.0±2.3 μM (FIG. **4**). This latter value is approximately 215-fold greater (i.e., less potent) than the EC_{50} value of 18.6±6.0 nM to cause coronary vasodilation (A_{2A} response-FIG. **3**).

The results indicate that Compound 16 is a coronary vasodilator (A_{2A} AcdoR-mediated effect) devoid of negative dromotropic effect (A_1 AdoR-mediated effect) in rat hearts. In guinea pig hearts Compound 16 caused some negative dromotropic effect. Nevertheless, Compound 16 was at least 215-fold more selective to cause coronary vasodilation than negative dromotropic effect. The reason(s) for the species difference in the A_1 AdoR-mediated response elicited by Compound 16 is unknown. Regardless, in both species (rat and guinea pig) Compound 16 causes maximal coronary vasodilation at concentrations that do not cause prolongation of A-V nodal conduction time, i.e., without negative dromotropic effect. It was also observed that Compound 16 has a greater affinity (i.e., >2-/>-13-fold) for A_{2A} than A_1 AdoR and that there is a markedly greater receptor reserve for A_{2A} AdoR-mediated coronary vasodilation than for A_1 AdoR-mediated negative dromotropic effect.

EXAMPLE 18

The present study was designed to test the hypothesis that there is an inverse relationship between the affinity (K_i or pK_i) and duration of action of A_2 adenosine receptors (AdoR). Specifically, the aims of the study were to determine the relationship between the duration of the coronary vasodilation caused by a selected series of high and low affinity A_{2A} AdoR agonists in rat isolated hearts and anesthetized pigs; and the affinity of these agonists for A_{2A} doRs in pig striatum.

Materials: Rats (Sprague Dawley) were purchased from Simonen. Farm pigs were obtained from Division of Laboratory Animal Resources, University of Kentucky. Compound 12, Compound 13, and Compound 16 of this invention were prepared as described in the methods above. YT-0146 was prepared as described in U.S. Pat. No. 4,956, 345, the specification of which is incorporated herein by reference. WRC-0470 was prepared as described in the literature (K. Niiya et al., J. Med. Chem. 35; 4557–4561 (1992). CGS21680 was purchased from Research Biochemicals, Inc. and Sigma and R-PIA (Lot No. WY-V-23) was purchased from Research Biochemicals, Inc. HENECA was a gift from Professor Gloria Cristalli of University of Camerino, Italy.

The anesthetic agents: Ketamine was purchased from Fort Dodge Animal Health. Xylazine was purchased from Bayer. Sodium pentobarbital was purchased from The Butler Co. Phenylephrine was purchased from Sigma DMSO was purchased from Sigma and American Tissue Type Collections. Krebs-Henseleit solution was prepared according to standard methods, and 0.9% saline was purchased from McGraw, Inc.

In this study, the following laboratory preparations were used. 1) Rat isolated perfused hearts; 2) Anesthetized open-chest pigs;

Rat Isolated Perfused Heart Preparation

Adult Sprague Dawley rats of either sex weighing from 230 to 260 grams were used in this study. Animals were anesthetized by peritoneal injection of a cocktail containing ketamine and xylazine (ketamine 100 mg, xylazine 20 mg/ml). The chest was opened and the heart quickly removed. The heart was briefly rinse in ice-cold Krebs-

Henseleit solution (see below), and the aorta cannulated. The heart was then perfused at a flow rate of 10 ml/min with modified Krebs-Henseleit (K-H) solution containing NaCl 117.9, KCl 4.5, CaCl. 2.5, $MgSO_4$1.18, KH2PO4 1.18, pyruvate 2.0 mmo/L. The K-H solution (pH 7.4) was gassed continuously with 95% $_2$ and 5% CO_2 and warmed to 35±0.50° C. The heart was electrically paced at a fixed cycle length of 340 ms (250 beats/min) using a bipolar electrode-place on the left atrium. The electrical stimuli were generated by a Grass stimulator (Model S48, W. Warwick, R.I.) and delivered through a Stimuli Isolation Unit (Model SIU5, Astro-Med, Inc., N.Y.) as square-wave pulses of 3 msec in duration and amplitude of at least twice the threshold intensity.

Coronary perfusion pressure (CPP) was measured using a pressure transducer, connected to the aortic cannula via a T-connector positioned approximately 3 cm above the heart Coronary perfusion pressure was monitored throughout the experiment and recorded either on a chart recorder (Gould Recorder ^{2200}S) or a computerized recording system (PowerLab/4S, ADInstruments Pty Ltd, Australia). Only hearts with CPP ranging from 60 to 85 mmHg (in the absence of drugs) were used in the study. Coronary conductance (in ml/min/mmHg) was calculated as the ratio between coronary perfusion rate (10 ml/min) and coronary perfusion pressure.

Anesthetized Open-chest Pig Preparation

Farm pigs weighing 22–27 kg were used in this study. All animals received humane care according to the guidelines set forth in The Principles of Laboratory Animal Care formulated by the National Society for Medical research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86–23, revised 1996). In addition, animals were used in accordance with the guidelines of the University of Kentucky Institutional Animal Care and Use Protocol.

Anesthesia was anesthetized with ketamine (20 mg/kg, i.m.) and sodium pentobarbital (15–18 mg/kg i.v.). Anesthesia was maintained with additional sodium pentobarbital (1.5–2 mg/kg, i.v.) every 15–20 minutes. Ventilation was maintained via a tracheotomy using a mixture of room air and 100% 02. Tidal volume, respiratory rate and fraction of $_2$ in inspired air were adjusted to maintain normal arterial blood gas (ABG) and pH values. Core body temperature was monitored with an esophageal temperature probe and maintained with a heating pad between 37.0–37.5° C. Lactate Ringers solution was administered via an ear or femoral vein, at 5–7 ml/kg/min after a initial bolus of 300–400 ml. A catheter was inserted into the femoral artery to monitor arterial blood pressure and to obtain ABG samples.

The heart was exposed through a median stemotomy, and suspended in a pericardial cradle. Left ventricular pressure (LVP) was measured with a 5F high fidelity pressure sensitive tip transducer (Millar Instrunents, Houston, Tex.) placed in the left ventricular cavity via the apex and secured with a purse string suture. A segment of the left anterior descending coronary artery (LAD), proximal to the origin of the first diagonal branch, was dissected free of, surrounding tissue. A transit time perivascular flow probe (Transonic Systems Inc., Ithaca, N.Y.) was placed around this segment to measure coronary blood flow (CBF). Proximal to the flow probe a 24 g modified angiocatheter was inserted for intracoronary infusions. All hemodynic data were continuously displayed on a computer monitor and fed through a 32 bit analogdigital converter into an on line data acquisition computer with customized software (Augury, Coyote Bay Instruments, Manchester, N.H.). A_{2A} AdoR agonists were dissolved in DMSO to produce stock concentrations of 1–5 mM, which were diluted in 0.9% saline and infused at rates of 1–1.5 ml/min. The A_{2A} AdoR agonists were administered intracoronary. To maintain blood pressure constant, phenylephrine was administered intravenously. The phenylephrine stock solution (30 mM) was prepared in distilled water.

Isolated Perfused Hearts

To determine the duration of the A_{2A} adenosine receptor mediated coronary vasodilation caused by adenosine and adenosine receptor agonists, the agonists were administered interveneously either by bolus injection (protocol A) or by continuous infusion (protocol B).

Protocol A: Bolus injections. In each heart of this series (3 to 11 hearts for each agonist), boluses of adenosine (20 μl, 2×10^{-4} M), Compounds of this invention (20 to 40 μl, 2×10^{-5} M), and other adenosine receptor agonists were injected into the perfusion line. The times to 50% (t 0.5) and 90%/o (t 0.9) reversal of the decrease in CPP were measured. Each heart was exposed to a maximum of three vasodilators.

Protocol B: Continuous infusion. In a separate series of experiments (n=4), Compound 16 and adenosine were infused into the perfusion line at constant rate for a period of six minutes. The perfusate concentrations of Compound 16 and adenosine were 20 nM and 200 nM respectively, which were approximately 4× their respective concentrations previously established to cause 50% of maximal increase in coronary conductance (EC_{50}) in rat isolated perfused hearts. The times to 50% (t 0.5) and 90% (t 0.9) reversal of the decreases in CPP were measured from the time at which the infusion of the agonists was stopped.

Dose-dependent duration of maximal vasodilation caused by bolus injections of Compound 16. To determine the dependency of the duration of maximal coronary vasodilation on the dose of Compound 16, boluses (100–300 μl) of a 2×10^{-5} M stock solution of Compound 16 were injected into the perfusion line. In addition, the duration of the injection was varied according to the volume of the boluses such as 10, 20 and 30 sec for 100, 200 and 300 μl boluses respectively. The duration of maximal effect was measured from the point at which the decrease in CPP reached the nadir to the onset point of reversal of CPP.

Relationship between affinity of various agonists for A_{2A} adenosine receptor and the reversal time of their effect to increase coronary conductance: These experiments were performed to construct the relationship between the affinities of the various agonists for A_{2A}adenosine receptor and the duration of their respective effect on coronary conductance. Boluses of various agonists were injected into the perfusion line of rat isolated perfused hearts (n=4 to 6 for each agonist) and the time to 90% (t0.9) reversal of the decrease in CPP measured. The affinities of the various agonists for A_{2A} adenosine receptor was determined in pig striatum membranes using a radioligand binding assay, as described above. The reversal time (t0.9) of the decrease in CPP was plotted against their affinities (pK_i) for the A_{2A} adenosine receptor.

Open-chest Pig

Prior to initiating the experiment, a 30-minute stabilization period followed the completion of all instrumentation. After obtaining the baseline hemodynamic data the first intracoronary infusion of an A_{2A} ADOR agonist was initiated Infusions were maintained for 4–5 minutes to allow LAD CBF to reach a steady state, after which the infusion was terminated. The time to recovery of 50% (t 0.5) and 90% (t0.9) of baseline CBF were recorded. Ten to 15 minutes

after CBF returned to pre-drug values a second infusion with a different agonist was started. In preliminary studies it was found that the intracoronary infusion of adenosine agonists produced varying degrees of systemic hypotension, and hence, in all subsequent experiments, phenylephrine was administered intravenously. Hemodynamic measurements were made prior to and following the initiation of the phenylephrine infusion at dose of −1 μg/kg/min. The phenylephrine infision rate was adjusted during and following the infusions of the adenosine agonists to maintain arterial blood pressure within 5 mmHg of preinfusion values. The effect of a maximum of three different agonists was determined in each experiment.

Results

Adenosine, the compounds of this invention and other adenosine derivatives were given as boluses into the perfusion line at concentrations that cause equal or near-equal increases in coronary conductance. Although adenosine and the agonists caused equal maximal increases in coronary conductance the duration of their effect was markedly different. The duration of the effect of adenosine was the shortest followed by Compound 16, whereas that of CGS21680 and WRC6470 were the longest. The durations of the coronary vasodilation caused by adenosine, the compounds of this invention and other agonists measured as the time to 50% and 90% (t 0.5 and t 0.9, respectively) reversal of the increases in coronary conductance are summarized in Table 4

TABLE 4

Reversal Time Of Coronary Vasodilation by Adenosine and adenosine receptor agonists in Rat Isolated Perfused Hearts

Agonist	t 0.5 (min)	t 0.9 (min)	n
Adenosine	1.06 ± 0.1	5.6 ± 0.8	11
HENECA	28.6 ± 1.1	32.8 ± 2.1	3
R-PIA	7.9 ± 0.1	12.6 ± 0.8	3
CGS21680	14.5 ± 0.9	19.5 ± 0.9	3
YT-146	17.7 ± 1.0	28.5 ± 4.0	3
Compound 12	14.83 ± 2.1	15.0 ± 0.8	3
Compound 13	14.4 ± 1.9	21.3 ± 3.9	4
Compound 16	5.2 ± 0.2	11.3 ± 1.1	5

Time (in minutes) to 50% and 90% (t 0.5 and t 0.9, respectively) reversal of the increases in coronary conductance caused by adenosine and adenosine receptor agonists. Values are the means ± SEM of single determinations in each of the preparations (n).

The reversal time of coronary vasodilation was dependent on the affinity of the adenosine derivatives for brain striatum A_{2A} receptors. (FIG. 2A) There was a significant ($P<0.05$) inverse relationship ($r=0.87$) between the affinity (PKi) of the agonists for the A_{2A} AdoR and the reversal time (t 0.9) of the coronary vasodilation caused by the same agonists.

Regardless of whether Compound 16 was given as bolus or continuous infuision the reversal of the coronary vasodilation was relatively rapid. In fact, a comparison between a six minute infusion of adenosine and Compound 16 at doses that they cause equal decreases in coronary perfusion pressure (CPP) revealed that adenosine and Compound 16 have a similar time course for vasodilation and reversal time. Both the t 0.5 and t 0.9 were near identical. The duration of the coronary vasodilation by Compound 16 was dose-dependent. Increasing the volume of a bolus of Compound 16 (stock solution of 2×10^{-5} M) caused progressively longer lasting decreases in CPP. The maximal duration of the coronary vasodilation (time that CPP remained at its lowest) increased as the volume of the boluses increased from 100 μl to 200 and 300 μl without affecting the maximal decreases in CPP.

Coronary Vasodilation in an Open-chest Pig Preparation

In in situ hearts of an open-chest anesthetized pig preparation Compound 16 of this invention as well as CGS21680 and other A_{2A}AdoR agonists (i.e., WRC-0470 and YT-146) caused significant increases in coronary blood flow (CBF). Selected doses of these compounds given as continuous (4 to 5 min) intracoronary infusions caused 3.1 to 3.8-fold increases in CBF as set forth in Table 3, below. Once established that all agonists caused near the same magnitude of increases in CBF (i.e., "fold increase") and cause similar changes in heart rate and mean arterial blood pressure, the reversal time of their respective coronary vasodilation effects was determined.

TABLE 5

Magnitude of Increase in Coronary Blood Flow Caused by Various Adenosine Receptor Agonists in Open-Chest Anesthetized Pigs

Agonist	CBF ("Fold Increase"	n
Compound 16 (10 μg/kg/min)	3.40 ± 0.04	3
Compound 16 (310 μg/kg/min)	3.83 ± 0.39	6
WRC-470 (1 μg/kg/min)	3.14 ± 0.24	6
GSC21680 (2 μg/kg/min)	3.54 ± 0.093	3
YT-146 (1 μg/kg/min)	3.44 ± 0.47	3

Maximal "fold-increase" in coronary blood flow (CBF) above baseline caused by various adenosine receptor agonists. Data represent mean ± SEM of one or two measurements in each pig (n).

As summarized in Table 6 the $t_{0.5}$ and $t_{0.9}$ of coronary vasodilation caused by the various A_{2A} AdoR agonists and "CVT-compounds" was variable. The reversal time of the increase in CBF caused by Compound 16 of this invention were shorter than that of CGS21680, WRC-0470 or YT-146. More importantly, as in rat isolated perfused hearts, there was a significant ($P<0.05$) inverse relationship ($r=0.93$) between the affinity (PKi) of the A_{2A} AdoR agonists for pig brain striatum A_{2A} receptors and the reversal time (t 0.9) of coronary vasodilation. There was an excellent concordance between the reversal time of the coronary vasodilation caused by a selected number of agonists in rat isolated perfused hearts and in anesthetized open chest pig preparations.

TABLE 6

Reversal Time of Coronary Vasodilation Caused by Various Adenosine Receptor Agonists in Open-Chest Anesthetized Pigs

Agonist	$t_{0.5}$ (min)	$t_{0.9}$ (min)	n
Compound 16 (10 μg/kg/min)	1.9 ± 0.2	10.1 ± 0.7	3
Compound 16 (310 μg/kg/min)	2.6 ± 0.4	12.3 ± 1.1	6
WRC-470 (1 μg/kg/min)	9.5 ± 0.8	22.5 ± 1.6	6
GS21680 (2 μg/kg/min)	9.7 ± 0.8	21.4 ± 0.8	3
YT-146 (1 μg/kg/min)	17.8 ± 3.4	32.9 ± 5.6	3

Time (in minutes) to 50% and 90% ($t_{0.5}$ and $t_{0.9}$ respectively) reversal of the increases in coronary blood flow caused by adenosine receptor agonists. Values are the means ± SEM of one or two determinations in each animal (n).

Compound 16 is a low affinity A_{2A} AdoR agonists and less potent (−10-fold) than the prototypical agonist CGS21680. Nevertheless Compound 16 is a full agonist to cause coronary vasodilation. But, as shown in this study the duration of its effect is several-fold shorter than that of the high affinity

agonists CGS21680 and WRC-0470. Hence, Compound 16 is a short acting A_{2A} AdoR agonists coronary vasodilator. Because of its short duration of action in comparison to the high affinity A_{2A} AdoR agonists (e.g., WRC-0470, CGS21680) this low affinity but still full agonist coronary vasodilator may prove to be ideal pharmacological "stressor agents" during radionuclide imaging of the myocardium.

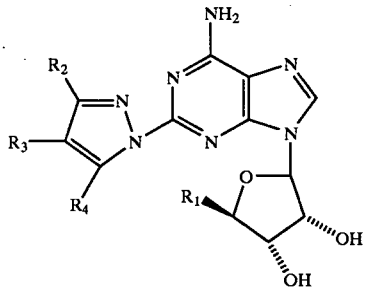
Experimental Reagents

Table 8 lists the A_{2A} adenosine agonists and antagonists that were used in Example s 14–18.

Compound	Abbreviation	Activity
5'-N-ethylcarboxamidoadenosine	NECA	A_{2A} agonist
N-[(1R)-1-methyl-1\2-phenyl-ethyl]adenosine 8-cyclopentyl-1, 3-dimethylxanthinge	R-PIA CPX	A_{2A} agonist A_{2b} antagonist
4-[2-[[6-Amino-9-(ethyl-B-D ribofuranuron-amindosyl)-9H-purin-2-yl]aminoethyl]benz-enepropanoic acid	CGS21680	A_{2A} adenosine receptor agonist
N-ethyl-1'-deoxy-1'-(6-amino-2-hexynyl-9H-purin9-yl)-beta-D-ribofura nuronamide	HENECA	A_{2a} adenosine receptor agonist
2-alkynyladenosine	YT-0146	A_{2a} adenosine receptor agonist
2-cyclohexylmethylidenehydrazino-adenosine	WRC0470	receptor agonist
4-(2-[7-amino-2-(2-furyl)[1,2,-4]triazolo[2,3-a] [1,3,5]triazin-5-ylamino]ethyl)phenol	ZM241385	A_{2A} adenosine receptor antagonist

What we claim is:

1. A compound having the formula:



wherein R^1=CH_2OH;

R^3 is selected from the group consisting of CO_2R^{20}, —$CONR^7R^8$, and aryl, wherein the aryl substituent is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, alkyl, and OR^{20};

R^7 is selected from the group consisting of hydrogen, straight or branched C_{1-15} alkyl and C_{3-8} cycloalkyl, wherein the alkyl substituent is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of aryl and CO_2R^{20}, and wherein the optional aryl substituent is optionally substituted with halo;

R^8 is selected from the group consisting of hydrogen, straight or branched C_{115} alkyl and C_{3-8} cycloalkyl;

R^{20} is selected from the group consisting of hydrogen and C_{1-15} alkyl;

and wherein R^2 and R^4 are hydrogen.

2. The compound of claim 1 wherein R^3 is CO_2R^{20}; and R^{20} is selected from the group consisting of hydrogen and C_{1-4} alkyl.

3. The compound of claim 1 wherein R^3 is $CONR^7R^8$;

R^7 is selected from the group consisting of hydrogen, straight or branched C^{1-10} alkyl and C_{3-5} cycloalkyl, wherein the alkyl substituent is optionally substituted with from 1 to 2 substituents independently selected from the group consisting of aryl and CO_2R^{20};

R^8 is selected from the group consisting of hydrogen, straight and branched C_{1-3} alkyl and C_{3-5} cycloalkyl; and

R^{20} is selected from the group consisting of C_{1-4} alkyl.

4. The compound of claim 1 wherein R^3 is aryl, wherein the aryl substituent is optionally substituted with from 1 to 3 substituents independently selected from the group consisting of halo, alkyl and OR^{20}; and

R^{20} is selected from and the group consisting of C^{1-4} alkyl.

5. The compound of claim 2 wherein R^3 is CO_2R^{20}; and

R^{20} is selected from the group consisting of hydrogen and C^{1-4} alkyl.

6. The compound of claim 3 wherein R 7 is selected from the group consisting of hydrogen, C_{1-3} alkyl and cyclopentyl, wherein the alkyl substituent is optionally substituted with from 1 to 2 substituents, independently selected from the group consisting of phenyl and CO_2R^{20} and wherein each optional phenyl substituent is optionally substituted with halo;

R^8 is selected from hydrogen and methyl; and

R^{20} is selected from hydrogen and ethyl.

7. The compound of claim 4 wherein

R^3 is aryl, wherein the aryl substituent is phenyl optionally substituted with from 1 to 2 substituents independently selected from the group consisting of chloro, methyl and OR^{20};

and R^{20} is methyl.

8. The compound of claim 1 selected from the group consisting of ethyl 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylate;

(4S,2R,3R,5R)-2-{6-amino-2-[4-(4-chlorophenyl)-pyrazolyl]purin-9-yl}-5-(hydroxymethyl)oxolane-3,4-diol;

(4S,2R,3R,5R)-2-{6-amino-2-[4-(4-methoxyphenyl) pyrazolyl]purin-9-yl}-5-(hydroxymethyl)oxolane-3,4-diol;

(4S,2R,3R,5R)-2-{6-amino-2-[4-(4-methylphenyl) pyrazolyl]purin-9-yl}-5-(hydroxymethyl)-oxolane-3,4-diol;

(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide;

1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxyrethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxylic acid;

(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N,N-dimethylcarboxamide;

(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-ethylcarboxamide;

1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazole-4-carboxamide;

1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-^{4}-yl)-N-(cyclopentyl)carboxamide;

(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-[(4-chlorophenyl)methyl]carboxamide, and

ethyl 2-[(1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl)oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)carbonylamino]acetate.

9. A method for stimulating coronary vasodilation in a mammal comprissing administering by intravenous bolus injection an amount of a compound of claim **1** that is sufficient to stress the heart and induce a coronary steal situation for the purposes of imaging the heart.

10. The method of claim **9** wherein the mammal is a human.

11. A pharmaceutical composition comprising a compound of claim **1** and one or mode pharmaceutical excipients.

12. The pharmaceutical composition of claim **11** wherein the pharmaceutical composition is in the form of a solution.

13. The compound of claim **8** wherein the compound is (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-methylcarboxamide.

14. The compound of claim **8** wherein the compound is 1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-(cyclopentyl)carboxamide.

15. The compound of claim **1** wherein the compound is (1-{9-[(4S,2R,3R,5R)-3,4-dihydroxy-5(hydroxymethyl) oxolan-2-yl]-6-aminopurin-2-yl}pyrazol-4-yl)-N-ethylcarboxamide.

16. A method of dilating the coronary vessels of a mammal, as an adjunct to angioplasty, with the pharmaceutical composition of claim **11**.

17. A method for adjunctive therapy in conjunction with angioplasty in a mammal comprising administering to the mammal a therapeutically effective amount of a compound of claim **1**.

18. A method for inhibition of platelet aggregation in a mammal comprising administering to the mammal a therapeutically effective amount of a compound of claim **1**.

* * * * *

ATTACHMENT B
COPIES OF THE CERTIFICATES OF CORRECTION WHICH ISSUED WITH RESPECT TO US PATENT NO. 6,642,210

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,642,210 B1
DATED : November 4, 2003
INVENTOR(S) : Zablocki et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 33,
Line 61, delete "C_{115}" and replace with -- $C_{1\text{-}15}$ --.

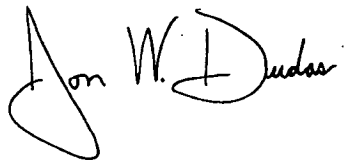
Column 34,
Line 3, delete "$C^{1\text{-}10}$" and replace with -- $C_{1\text{-}10}$ --.
Lines 16 and 19, delete "$C^{1\text{-}14}$" and replace with -- $C_{1\text{-}4}$ --.
Line 20, delete "R7" and replace with -- R^7 --.
Line 66, delete "pyrazol-^{4}-yl)" and replace with -- pyrazole-4-yl) --.

Column 35,
Line 8, delete "comprissing" and replace with -- comprising --.

Column 36,
Line 6, delete "claim 1" and replace with -- claim 8 --.

Signed and Sealed this

Thirteenth Day of January, 2004



JON W. DUDAS
Acting Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

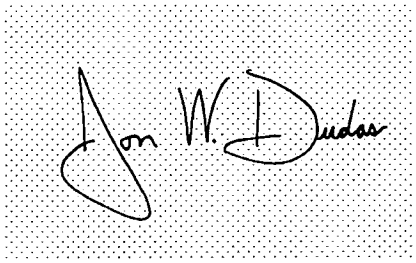
PATENT NO. : 6,642,210 B1
DATED : November 4, 2003
INVENTOR(S) : Zablocki et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 18,
Line 66, delete "(d, 1H), 4.34 (d, 1H), 4.64 (m, 1H), 6.06 (d, 1H), 8.38 (s," and replace with -- (d, 1H), 4.34 (d, 1H), 4.64 (m, 1H), 6.06 (d, 1H), 8.11 (s,1H), 8.38 (s, --.

Signed and Sealed this

Twenty-seventh Day of December, 2005



JON W. DUDAS
Director of the United States Patent and Trademark Office

ATTACHMENT C
NOTICE OF RECORDATION AND ASSIGNMENT FROM THE INVENTORS TO CV THERAPEUTICS, INC.

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

500005991A

SEPTEMBER 02, 2004

PTAS

MCDONNELL BOEHNEN HULBERT & BERGHOFF LLP
300 S. WACKER DRIVE
A. BLAIR HUGHES
CHICAGO, IL 60606

UNITED STATES PATENT AND TRADEMARK OFFICE
NOTICE OF RECORDATION OF ASSIGNMENT DOCUMENT

THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

PLEASE REVIEW ALL INFORMATION CONTAINED ON THIS NOTICE. THE INFORMATION CONTAINED ON THIS RECORDATION NOTICE REFLECTS THE DATA PRESENT IN THE PATENT AND TRADEMARK ASSIGNMENT SYSTEM. IF YOU SHOULD FIND ANY ERRORS OR HAVE QUESTIONS CONCERNING THIS NOTICE, YOU MAY CONTACT THE EMPLOYEE WHOSE NAME APPEARS ON THIS NOTICE AT 703-308-9723. PLEASE SEND REQUEST FOR CORRECTION TO: U.S. PATENT AND TRADEMARK OFFICE, ASSIGNMENT DIVISION, BOX ASSIGNMENTS, CG-4, 1213 JEFFERSON DAVIS HWY, SUITE 320, WASHINGTON, D.C. 20231.

RECORDATION DATE: 09/01/2004

REEL/FRAME: 015056/0942
NUMBER OF PAGES: 6

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
ZABLOCKI, JEFF A.

DOC DATE: 08/04/2004

ASSIGNOR:
ELZEIN, ELLFAITH O.

DOC DATE: 08/04/2004

ASSIGNOR:
PALLE, VENKATA P.

DOC DATE: 07/05/2004

ASSIGNEE:
CV THERAPEUTICS, INC.
3172 PORTER DRIVE
PALO ALTO, CALIFORNIA 94304

SERIAL NUMBER: 10018446
PATENT NUMBER: 6642210
TITLE: N-PYRAZOLE A2A RECEPTOR AGONISTS

FILING DATE: 04/12/2002
ISSUE DATE: 11/04/2003

KIMBERLY WHITE, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

PATENT ASSIGNMENT

Electronic Version v1.1
Stylesheet Version v1.1

09/01/2004
500005991

SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT

CONVEYING PARTY DATA

Name	Execution Date
Jeff A. Zablocki	08/04/2004
Ellfaith O. Elzein	08/04/2004
Venkata P. Palle	07/05/2004

RECEIVING PARTY DATA

Name:	CV Therapeutics, Inc.
Street Address:	3172 Porter Drive
City:	Palo Alto
State/Country:	CALIFORNIA
Postal Code:	94304

PROPERTY NUMBERS Total: 1

Property Type	Number
Patent Number:	6642210

CH $40.00 6642210

CORRESPONDENCE DATA

Fax Number: (312)913-0002
Correspondence will be sent via US Mail when the fax attempt is unsuccessful.
Phone: 312-913-0001
Email: hughes@mbhb.com
Correspondent Name: McDonnell Boehnen Hulbert & Berghoff LLP
Address Line 1: 300 S. Wacker Drive
Address Line 2: A. Blair Hughes
Address Line 4: Chicago, ILLINOIS 60606

NAME OF SUBMITTER:	A. Blair Hughes

Total Attachments: 4
source=99,423-S Assignment#page1.tif
source=99,423-S Assignment#page2.tif
source=99,423-S Assignment#page3.tif

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ASSIGNMENT

Case No.: 99,423-S
Inventors: Jeff A. Zablocki, Elfatih O. Elzein, and Venkata P. Palle
Date of Execution of Application: 2/27/02; 2/25/02; 2/19/02

Serial No.: 10/018,446
Patent No.: 6,642,210
Filing Date: April 12, 2000
Issue Date: Nov.4, 2003

In consideration of One Dollar ($1.00) and other good and valuable considerations in hand paid, the receipt and sufficiency whereof are hereby acknowledged, the undersigned hereby assign to:

CV Therapeutics, Inc.

its successors and assigns, the entire right, title and interest in the invention or improvements of the undersigned disclosed in an application for Letters Patent of the United States, entitled:

2-(N-PYRAZOLO) ADENOSINES WITH APPLICATION AS ADENOSINE A_{2A} RECEPTOR AGONISTS

and identified as:

Case No. 99,423-S

in the offices of McDONNELL BOEHNEN HULBERT & BERGHOFF and in said application and any and all other applications and corresponding patents, both United States and foreign, which the undersigned may file, either solely or jointly with others, on said invention or improvements, and in any and all Letters Patent of the United States and foreign countries, which may be obtained on any of said applications, and in any reissue or extension of such patents, and further assigns to said assignee the priority right provided by the International Convention.

The undersigned hereby authorize and request the Commissioner of Patents and Trademarks to issue said Letters Patent to said assignee.

The undersigned hereby authorize and request the attorneys of record in said application to insert in this assignment the filing date and serial number of said application when officially known, and the date of execution of the application.

The undersigned warrant themselves to be the owners of the entire right, title and interest in said invention or improvements and to have the right to make this assignment, and further warrant that there are no outstanding prior assignments, licenses, or other encumbrances on the interest herein assigned.

McDONNELL BOEHNEN
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001

For said considerations the undersigned hereby agree, upon the request and at the expense of said assignee, its successors and assigns, to execute any and all divisional, continuation and substitute applications for said invention or improvements, and any necessary oath, affidavit or declaration relating thereto, and any application for the reissue or extension of any Letters Patent that may be granted upon said application and any and all applications and other documents for Letters Patent in foreign countries on said invention or improvements, that said assignee, its successors or assigns may deem necessary or expedient, and for the said considerations the undersigned authorize said assignee to apply for patents for said invention or improvements in its own name in such countries where such procedure is proper and further agree, upon the request of said assignee, its successors and assigns, to cooperate to the best of the ability of the undersigned with said assignee, its successors and assigns, in any proceedings or transactions involving such applications or patents, including the preparation and execution of preliminary statements, giving and producing evidence, and performing any and all other acts necessary to obtain, maintain and enforce said Letters Patent, both United States and foreign, and vest all rights therein hereby conveyed in the assignee, its successors and assigns, whereby said Letters Patent will be held and enjoyed by the said assignee, its successors and assigns, to the full end of the term for which said Letters Patent will be granted, as fully and entirely as the same would have been held and enjoyed by the undersigned if this assignment had not been made.

WITNESS my hand and seal this 4 day of Aug, 2004.

Jeff A. Zablocki

Jeff A. Zablocki

State of California

County of Santa Clara

The foregoing instrument was acknowledged before me this 4th day of August, 2004 by JEFF ZABLOCKI

JOAN MAZZEO
Comm. # 1314298
NOTARY PUBLIC - CALIFORNIA
City & County of San Francisco
My Comm. Expires July 21, 2005

Joan Mazzeo

NOTARY PUBLIC

McDONNELL BOEHNEN
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001

WITNESS my hand and seal this 4 day of Aug, 2004.

Elfaith Elzein

Elfaith O. Elzein

State of California

County of Santa Clara

The foregoing instrument was acknowledged before me this 4th day of August, 2004 by ELFATIH ELZEIN

JOAN MAZZEO
Comm. # 1314298
NOTARY PUBLIC - CALIFORNIA
City & County of San Francisco
My Comm. Expires July 21, 2005

Joan Mazzeo

NOTARY PUBLIC

WITNESS my hand and seal this ___ day of _____, _____.

Venkata P. Palle

State of

County of

The foregoing instrument was acknowledged before me this _____ day of

_____, _____ by

NOTARY PUBLIC

McDONNELL BOEHNEN
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001

WITNESS my hand and seal this ___ day of _____, _____.

_____
Elfaith O. Elzein

State of

County of

The foregoing instrument was acknowledged before me this _____ day of

_____, _____ by

_____
NOTARY PUBLIC

WITNESS my hand and seal this 5th day of July, 2004.

_____
Venkata P. Palle

State of

County of

The foregoing instrument was acknowledged before me this _____ day of

, by

_____
NOTARY PUBLIC

McDONNELL BOEHNEN
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606

ATTACHMENT D
COPY OF THE TERMINAL DISCLAIMER FILED WITH RESPECT TO US PATENT NO. 6,403,567

PTO/SB/26 (10-00)
Approved for use 10/31/2002. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A PRIOR PATENT	Docket Number (Optional) 99,923-S

In re Application of: **Zablocki et al.**

Application No.: **10/018,446**

Filed: **April 12, 2002**

For: **N-Pyrazole A2A Receptor Agonists**

The owner*, **CV Therapeutics, Inc.**, of **100** percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application, which would extend beyond the expiration date of the full statutory term defined in 35 U. S. C. 154 to 156 and 173, as presently shortened by any terminal disclaimer, of prior Patent No. **6,403,567**. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

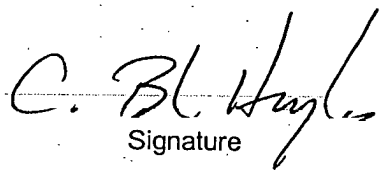
In making the above disclaimer, the owner does not disclaim the terminal part of any patent granted on the instant application that would extend to the expiration date of the full statutory term as defined in 35 U.S.C. 154 to 156 and 173 of the prior patent, as presently shortened by any terminal disclaimer, in the event that it later: expires for failure to pay a maintenance fee, is held unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

Check either box 1 or 2 below, if appropriate.

1. ☐ For submissions on behalf of an organization (e.g., corporation, partnership, university, government agency, etc.), the undersigned is empowered to act on behalf of the organization.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2. ☒ The undersigned is an attorney or agent of record.



Signature

February 25, 2003
Date

A. Blair Hughes
Typed or printed name

☒ Terminal disclaimer fee under 37 CFR 1.20(d) included.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner). Form PTO/SB/96 may be used for making this certification. See MPEP § 324.

ATTACHMENT E
COPY OF POWER OF ATTORNEY

PTO/SB/81 (01-06)
Approved for use through 12/31/2008. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

POWER OF ATTORNEY and CORRESPONDENCE ADDRESS INDICATION FORM

Application Number	10/018,446; Patent No. 6,642,210
Filing Date	21 Jun 2000; Issued 04 November 2003
First Named Inventor	Jeff Zablocki
Title	2-(N-Pyrazolo)Adenosines With
Art Unit	1623
Examiner Name	CRANE, LAWRENCE E.
Attorney Docket Number	99-0423-S

I hereby revoke all previous powers of attorney given in the above-identified application.

I hereby appoint:

[X] Practitioners associated with the Customer Number: 27716

OR

[ ] Practitioner(s) named below:

Name	Registration Number

as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

Please recognize or change the correspondence address for the above-identified application to:

[X] The address associated with the above-mentioned Customer Number:

OR

[ ] The address associated with Customer Number:

OR

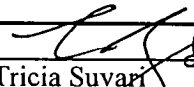
[ ] Firm or Individual Name					
Address					
City		State		Zip	
Country					
Telephone		Email			

I am the:

[ ] Applicant/Inventor.

[X] Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

SIGNATURE of Applicant or Assignee of Record

Signature		Date	6/3/08
Name	Tricia Suvari	Telephone	(650) 384-8500
Title and Company	Senior Vice President and General Counsel		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

[X] *Total of 2 forms are submitted.

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/96 (01-08)
Approved for use through 05/31/2008. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: CV Therapeutics, Inc.

Application No./Patent No.: 6,642,210 Filed/Issue Date: 4 November 2003

Entitled: 2-(N-Pyrazolo)Adenosines With Application As Adenosine A2A Receptor Agonists

CV Therapeutics, Inc., a Corporation
(Name of Assignee) (Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. [X] the assignee of the entire right, title, and interest; or

2. [ ] an assignee of less than the entire right, title and interest
(The extent (by percentage) of its ownership interest is _____%)

in the patent application/patent identified above by virtue of either:

A. [X] An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 015056, Frame 0942, or for which a copy thereof is attached.

OR

B. [ ] A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

[ ] Additional documents in the chain of title are listed on a supplemental sheet.

[ ] As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

Signature	6/3/08 Date
Tricia Suvari	(650) 384-8500
Printed or Typed Name	Telephone Number
Senior Vice President and General Counsel	
Title	